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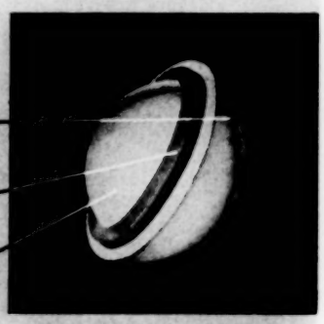
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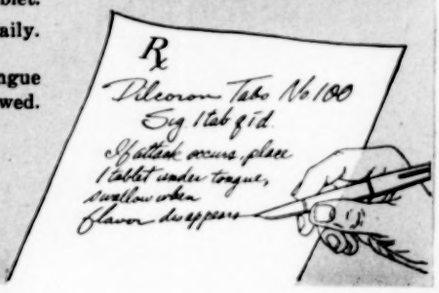


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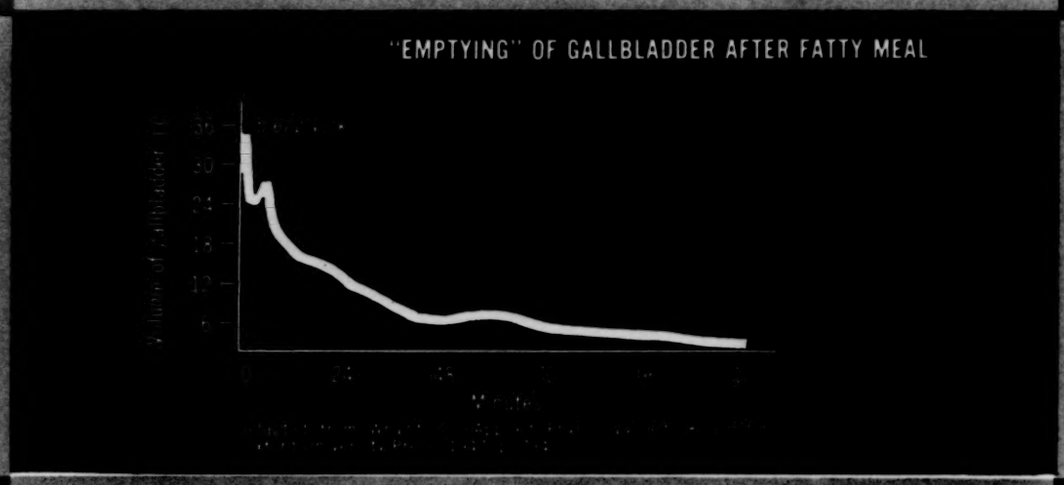
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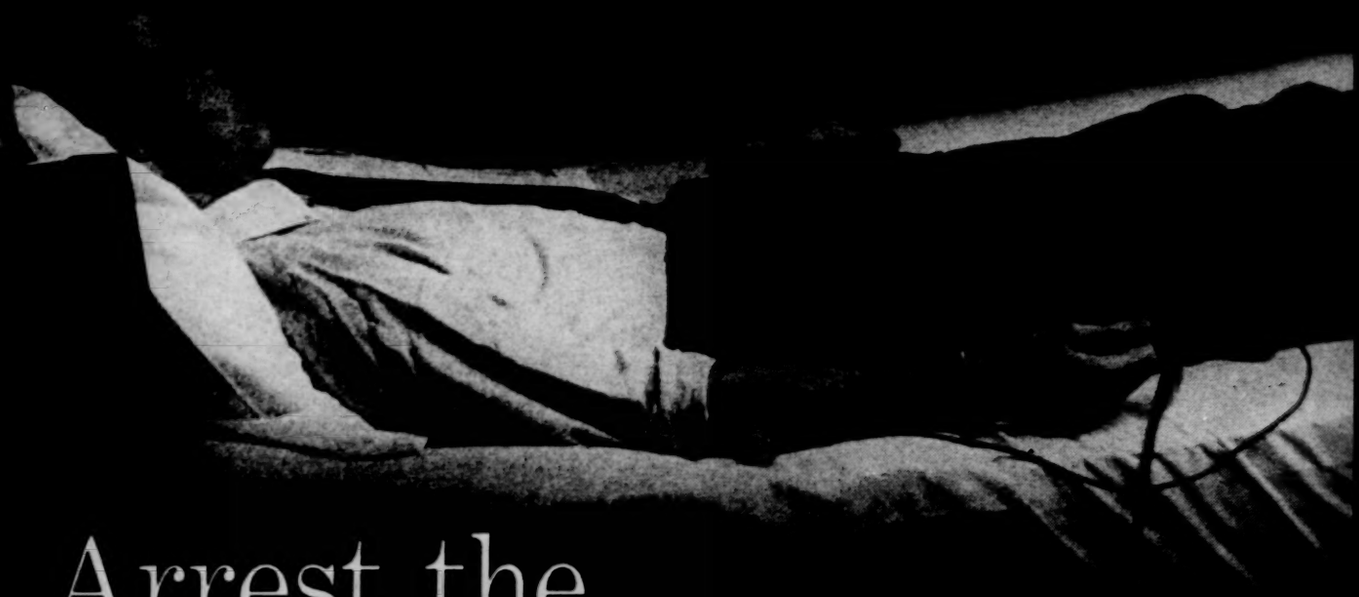
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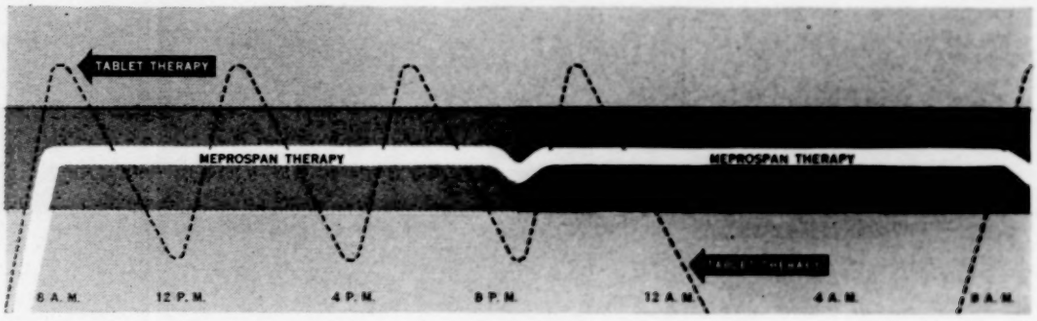
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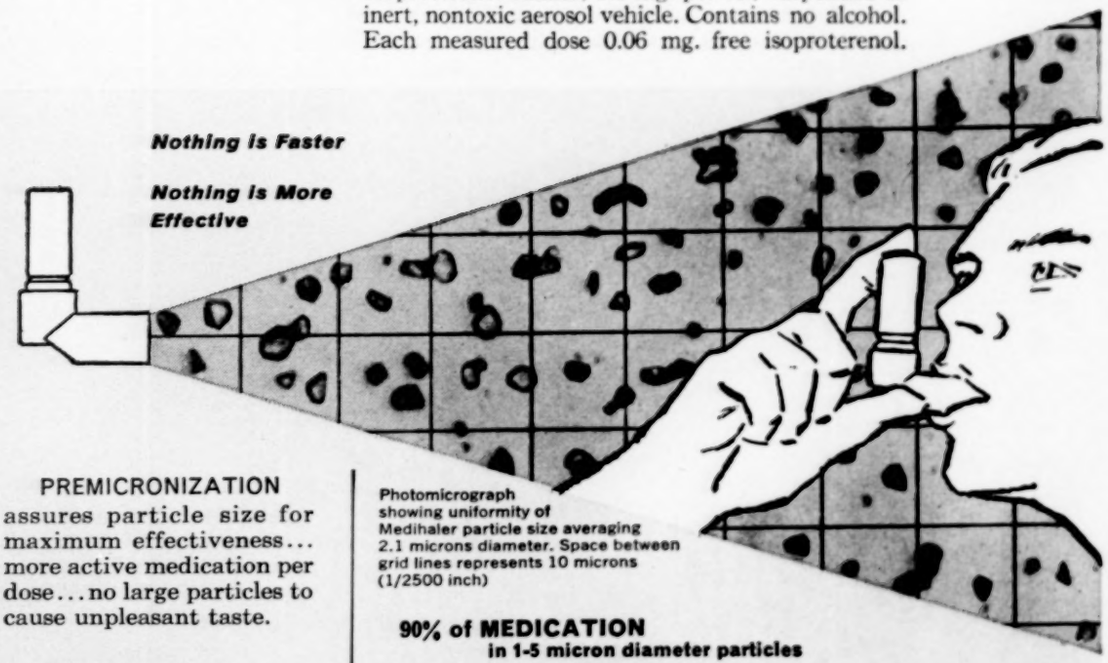
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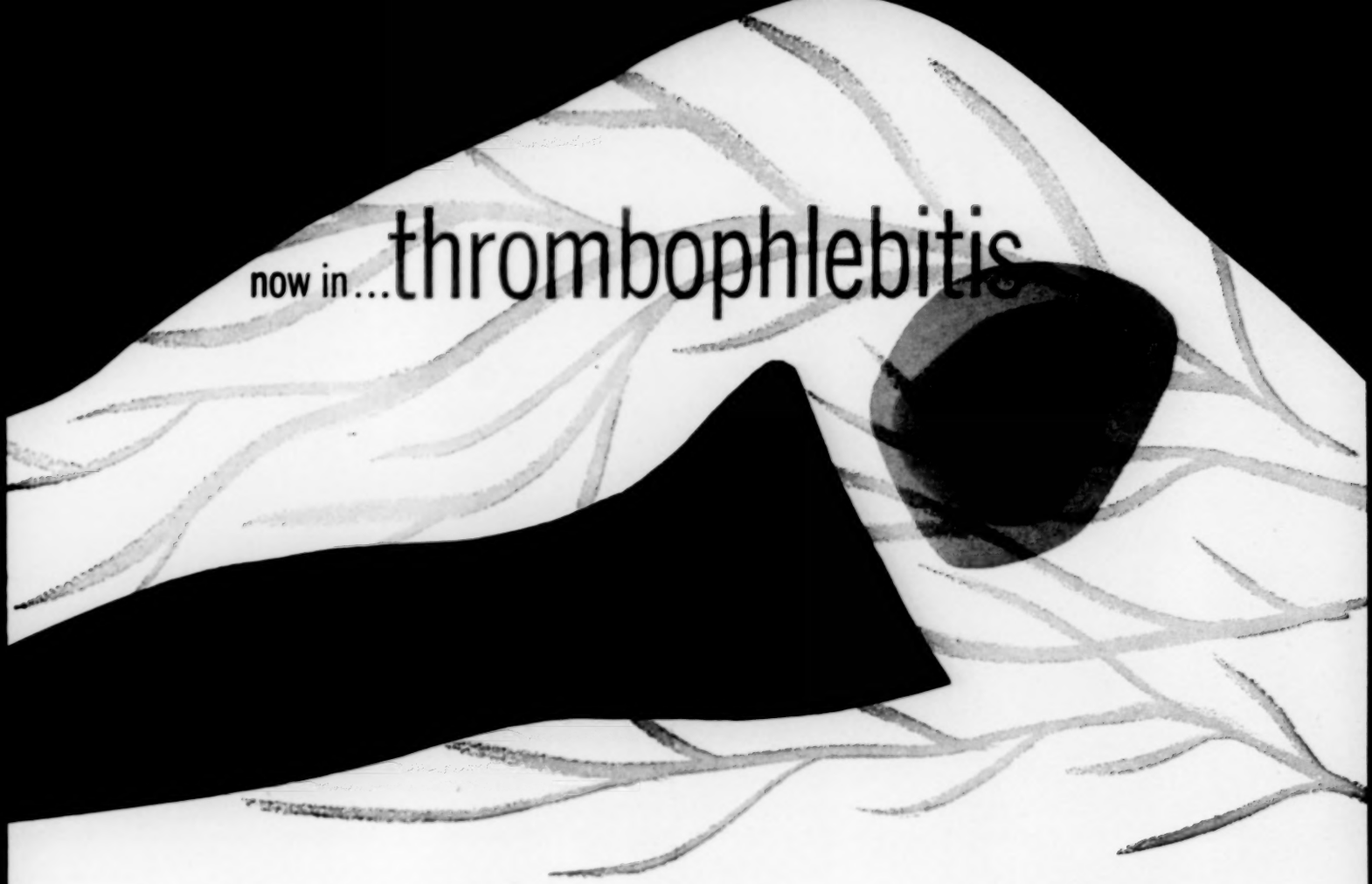
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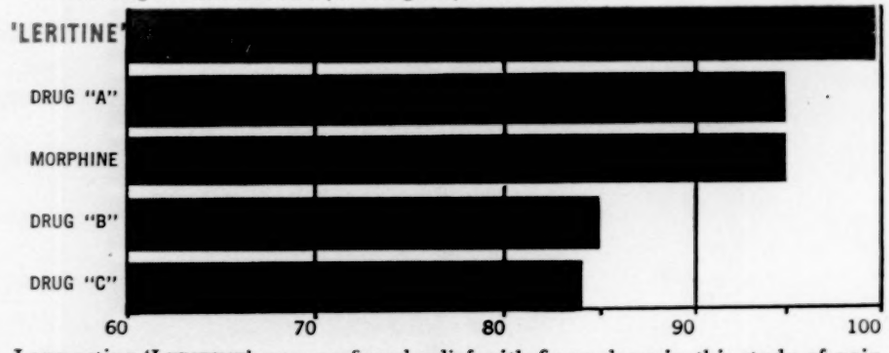
• In a series of 300 patients, anileridine, used as a primary anesthetic agent, "appeared to be more potent than meperidine, and with the drip method, no significant apnea or hypotension occurred.... Analgesia appeared to persist up to three hours postoperatively."[3]

• "In this study, anileridine was found to be two and one-half times as potent an analgesic as meperidine on a milligram basis."[4]

• In a comparative study of 278 patients 'LERITINE' gave profound relief with potency approaching that of morphine with minimal side effects.[5] This is illustrated in the following chart:

CHART 1: COMPARATIVE RELIEF OF POSTOPERATIVE PAIN (3-Day Period)

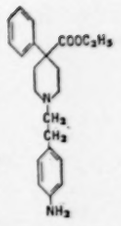
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1. Keesling, R., Hinds, E. C., and Keats, A. S.: Principles of Drug Evaluations in Dental Surgery: Antihistamines and Analgesics: (Paper read at the Thirty-fifth Gen. Meeting of the International Assoc. for Dental Research: Atlantic City, March 21-24, 1957). 2. Orahovats, P. D., Lehman, E. G., Chapin, E. W.: Pharmacology of Ethyl-1-(4-Aminophenethyl)-4-Phenylisonipecotate, Anileridine, A New Potent Synthetic Analgesic: Journal of Pharmacology and Experimental Therapeutics, Vol. 119, No. 1, (January) 1957. 3. Stage, J. T.: Anileridine as an Anesthetic Agent: Journal of the Florida Medical Association 44:143-145 (August) 1957. 4. Keats, A. S., Telford, J., Kurosu, Y.: Studies of Analgesic Drugs: Anileridine Dihydrochloride: Anesthesiology 18:690-697 (September-October) 1957. 5. Riffin, I. M., Preisig, R., Wheaton, H. H., Landman, M. E., and Schwarz, B. E.: "A New Synthetic Analgesic, Anileridine," Scientific Exhibit, 106th A.M.A. Annual Meeting, New York; June 3-7, 1957.

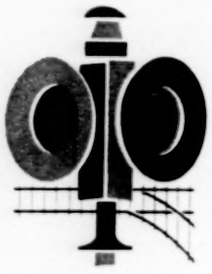
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"possesses outstanding qualities in terms of freedom from undesirable gastrointestinal effects."

"The chelation of iron minimized its toxicity and provided a high factor of safety against fatal poisoning."

AVAILABLE AS: CHĒL-IRON TABLETS BOTTLES OF 100 3 tablets supply 120 mg. elemental iron. CHĒL-IRON PEDIATRIC DROPS 30-CC. BOTTLES with graduated dropper each cc. supplies 16 mg. elemental iron; 0.5 cc. provides full M.D.R. for infants and children up to six. CHĒL-IRON PLUS TABLETS BOTTLES OF 100 3 tablets supply 72 mg. elemental iron plus B_{12} with intrinsic factor, folic acid, pyridoxine, other essential B vitamins, and C.

*Franklin, M., et al.: Chelate Iron Therapy, J.A.M.A. 166:1685, Apr. 5, 1958.
†U. S. Pat. 2,575,611

Kinney® KINNEY & COMPANY, INC. COLUMBUS, INDIANA



BUTIBEL®

has many unique advantages as an antispasmodic-sedative . . .

Butibel contains (per tablet or 5 cc.):

BUTISOL SODIUM® 10 mg. (⅙ gr.)
Butabarbital Sodium

"daytime sedative" with less risk of accumulation or development of tolerance.

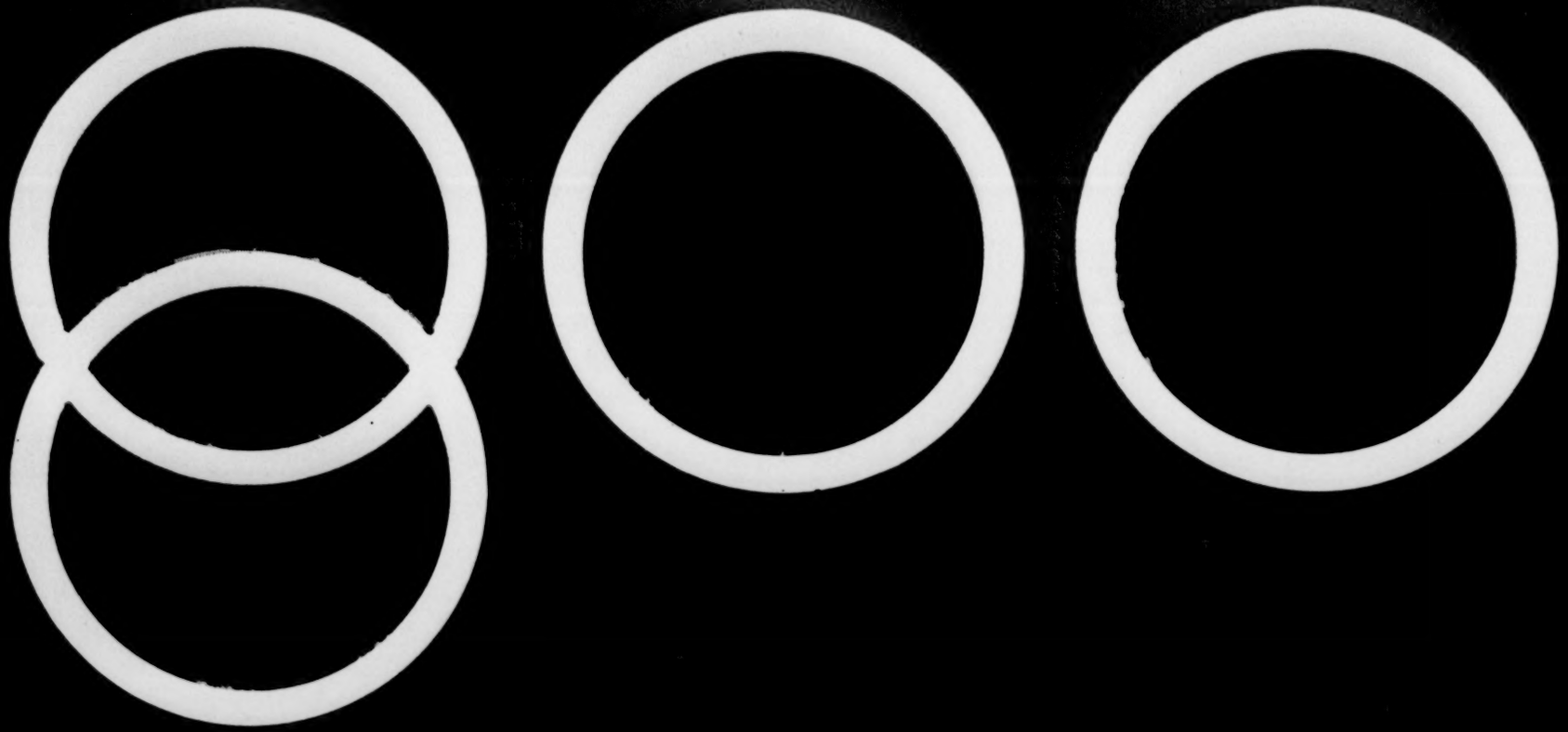
Ext. Belladonna 15 mg. (¼ gr.)

Natural belladonna and Butisol have approximately equal durations of action (no overlapping sedation or inadequate spasmolysis).

Butibel tablets . . . elixir . . .
Prestabs® Butibel R-A (Repeat Action Tablets)

McNEIL

LABORATORIES, INC.
Philadelphia 32, Pa.



a new specific

moniliacide MICOFUR™ and
brand of nifuroxime

the established specific

trichomonacide FUROXONE®
brand of furazolidone

in

TRICOFURON®

VAGINAL SUPPOSITORIES AND POWDER

■ **RAPID RELIEF OF BURNING AND ITCHING OFTEN WITHIN 24 HOURS**

■ **ELIMINATES MALODOR** ■ **ESTHETICALLY ACCEPTABLE, NON-IRRITATING**

85% CLINICAL CURES* In 219 patients with either trichomonal vaginitis, monilial vaginitis, or both, clinical cures were secured in 187.

71% CULTURAL CURES* 157 patients showed negative culture tests at 3 months' follow-up examinations.

Simple two-step treatment swiftly brings relief and control of vaginal moniliasis and trichomoniasis.



STEP 1 *Office* administration of TRICOFURON VAGINAL POWDER IMPROVED Applied by the physician at least once a week, except during menstruation.

(Micofur 0.5% [*anti* 5-nitro-2-furaldoxime], the new nitrofuran fungicide, and Furoxone 0.1% in an acidic, water-soluble powder base). Plastic insufflator of 15 Gm., with 3 sanitary disposable tips. Also glass bottle of 30 Gm.

STEP 2 Continued *home* use to maintain moniliacidal-trichomonacidal action: TRICOFURON VAGINAL SUPPOSITORIES IMPROVED Employed by the patient each morning and night the first week and each night thereafter—through one cycle, especially during the important menstrual days.

(Micofur 0.375% and Furoxone 0.25% in a water-soluble base).

Box of 12 wedge-shaped suppositories without applicator.

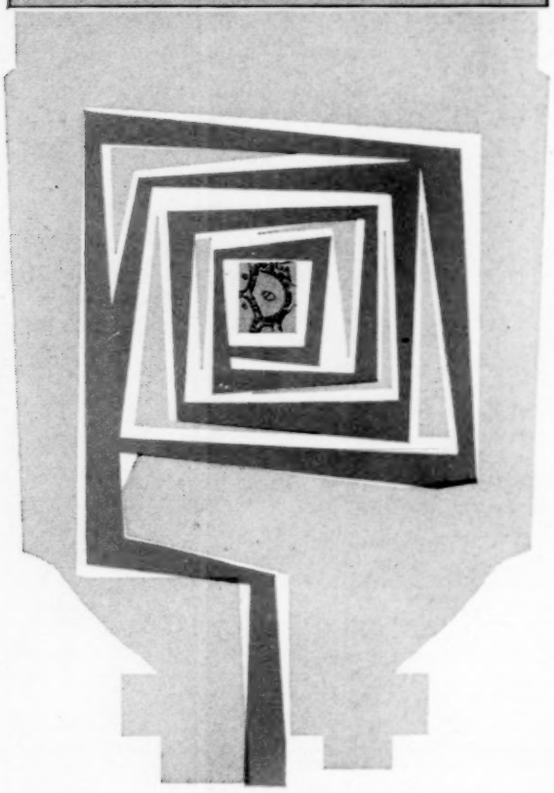
***Combined results of 12 clinical investigators. Data available on request.**



NITROFURANS—a *new* class of antimicrobials—neither antibiotics nor sulfonamides

EATON LABORATORIES, NORWICH, NEW YORK

AMIGEN 800 / *food to build*



"square meals in round bottles"

Amigen® 800

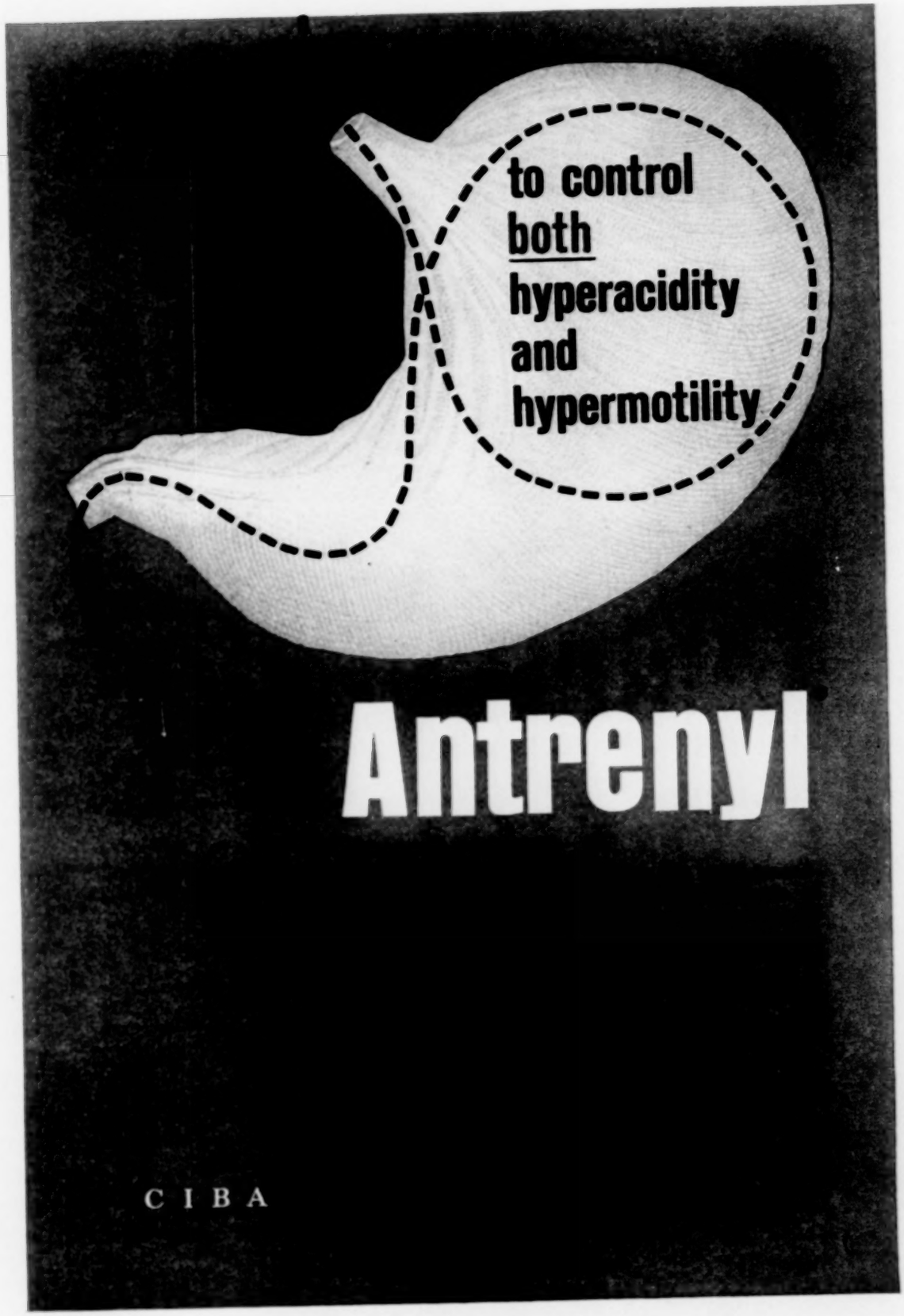
Amigen 5%, Levugen 12.5%, Alcohol 2.4%

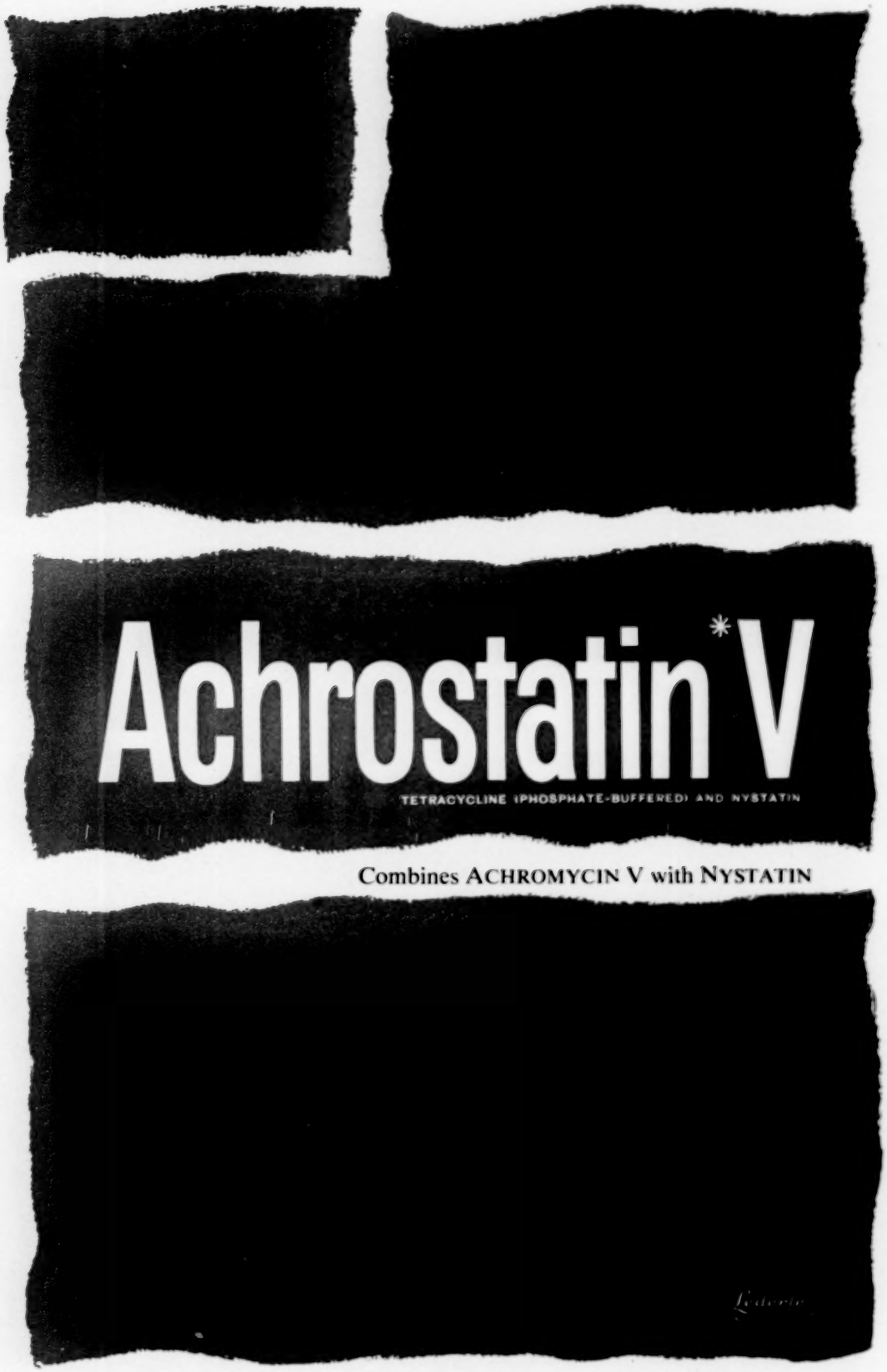
Amigen 800 provides the patient with more food per day—more of a "square meal"—*than any other parenteral solution.* It provides amino acids to build tissue, extra calories to spend for energy—and maintenance electrolytes and water. Patients receiving Amigen 800 usually maintain their weight and sometimes gain. *For details of the complete Mead Johnson Fluid Therapy Program write to Parenteral Division, Mead Johnson & Company, Evansville 21, Indiana.*

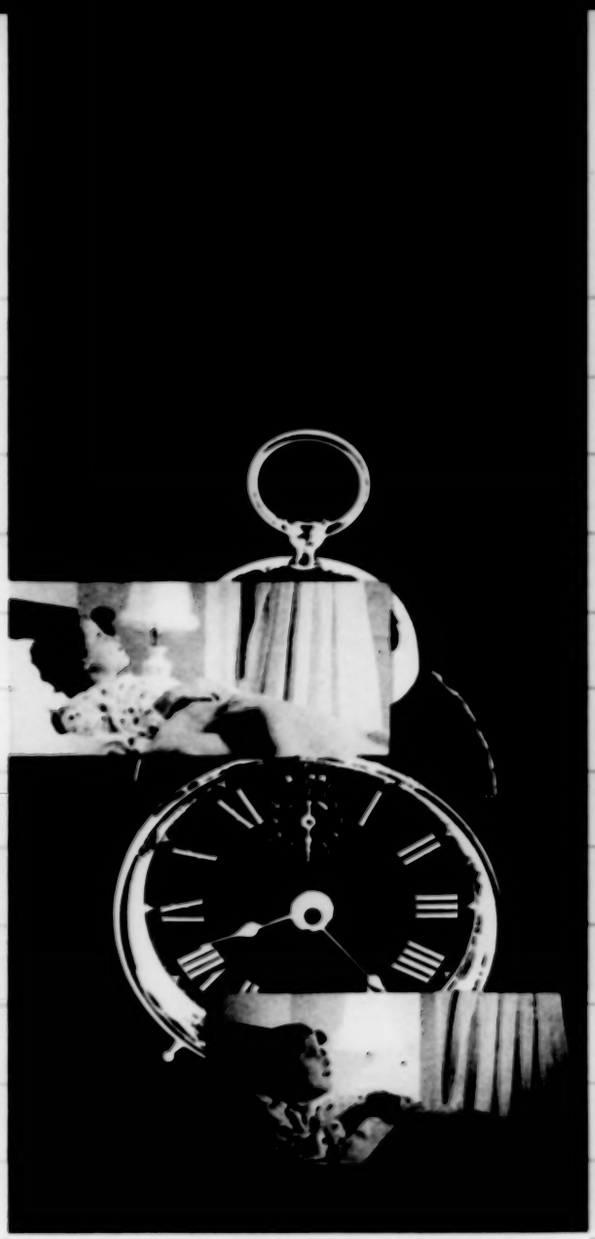
SOLUTIONS	*Standard · Amigen · Homeolytes · Levugens*
EQUIPMENT	*solution sets · blood sets and bottles*
SERVICES	*therapy guides · teaching aids*

Mead Johnson

Symbol of service in medicine


to control
both
hyperacidity
and
hypermotility
Antrenyl
CIBA


Achrostatin* V
TETRACYCLINE (PHOSPHATE-BUFFERED) AND NYSTATIN
Combines ACHROMYCIN V with NYSTATIN
Lederle



How many doses per day?

In sulfa therapy with Lipo Gantrisin, you determine the number of doses per day. Depending on the situation of the patient, and the persons responsible for his care, you can prescribe two, four, or six doses of Lipo Gantrisin per 24 hours. The prolonged, complete absorption phase of Lipo Gantrisin continues for up to 12 hours; you can maintain therapeutically effective lymph and urine levels, as well as adequate blood levels, on whatever regimen you choose.

Lipo Gantrisin

ROCHE

Roche Laboratories
Division of Hoffmann-La Roche Inc.
Nutley 10, N. J.

Lipo Gantrisin® Acetyl – brand of acetyl sulfisoxazole. Each teaspoonful (5 cc) contains the equivalent of 1 Gm Gantrisin (in the form of Gantrisin Acetyl) in a palatable, readily digestible homogenized mixture.

LIPO GANTRISIN

Antibacterial therapy with two doses daily

Description: With Lipo Gantrisin, therapeutic blood levels can usually be maintained 12 hours with a single dose. Lipo Gantrisin contains Gantrisin Acetyl emulsified in a readily digestible vegetable oil for higher and more prolonged blood levels. Each teaspoonful (5 cc) is equivalent to 1 Gm of sulfisoxazole (Gantrisin) in the form of acetyl sulfisoxazole.

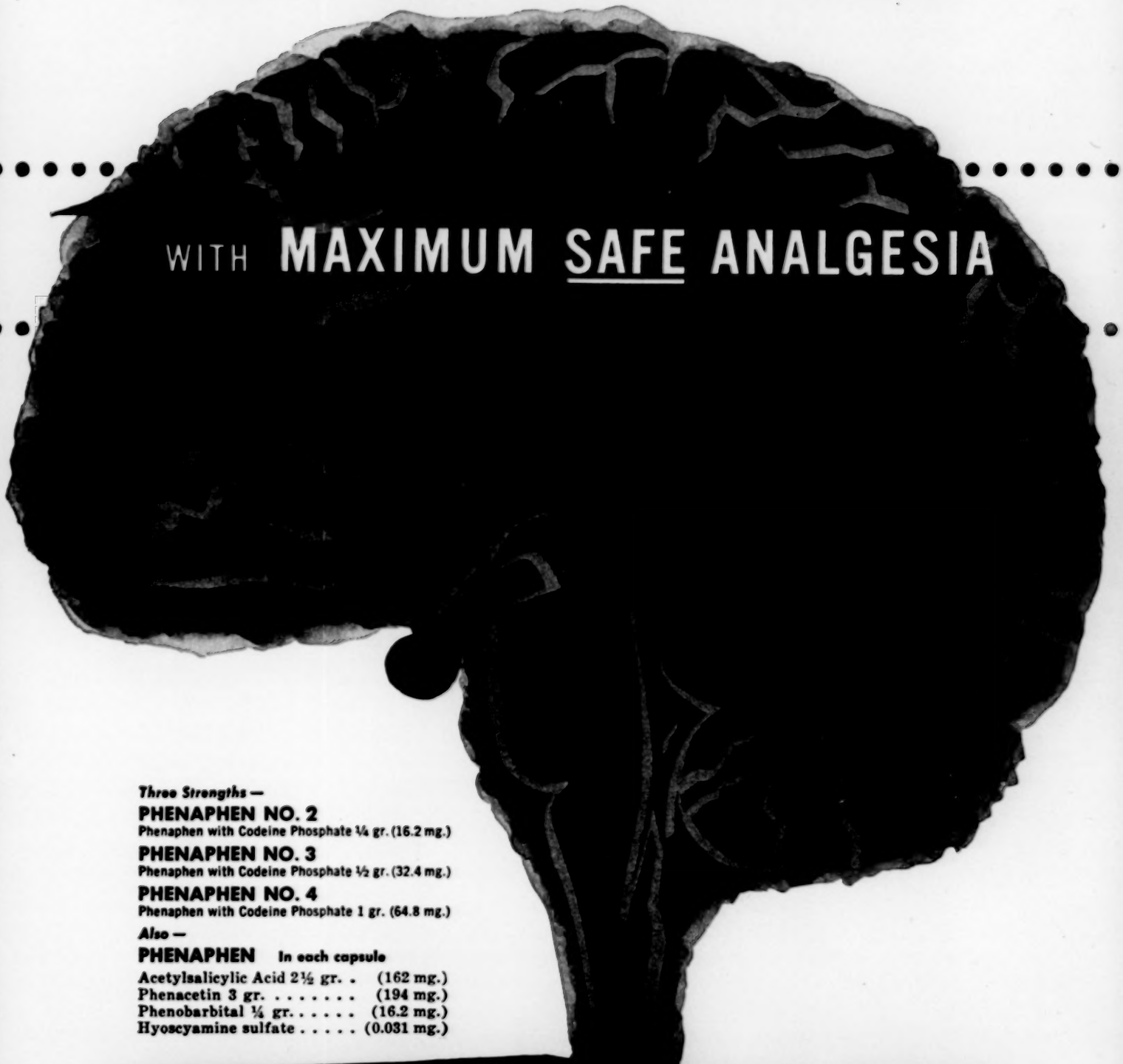
Properties: Two doses a day of Lipo Gantrisin are adequate. Like Gantrisin, Lipo Gantrisin can be employed without alkalies and with little danger of renal blocking.

Indications: Systemic and urinary tract infections due to susceptible micro-organisms.

Advantages: Dosage schedule adjustable to the patient's requirements . . . higher, more prolonged blood levels . . . wide antibacterial spectrum . . . virtually no danger of renal blocking . . . no need for alkalies . . . especially convenient for children.

For dosage and supply refer to PDR, page 760.

Raise the Pain Threshold



WITH MAXIMUM SAFE ANALGESIA

Three Strengths —

PHENAPHEN NO. 2
Phenaphen with Codeine Phosphate ¼ gr. (16.2 mg.)

PHENAPHEN NO. 3
Phenaphen with Codeine Phosphate ½ gr. (32.4 mg.)

PHENAPHEN NO. 4
Phenaphen with Codeine Phosphate 1 gr. (64.8 mg.)

Also —

PHENAPHEN In each capsule

Acetylsalicylic Acid 2½ gr. . (162 mg.)
Phenacetin 3 gr. . . . . (194 mg.)
Phenobarbital ¼ gr. . . . . (16.2 mg.)
Hyoscyamine sulfate . . . . . (0.031 mg.)

PHENAPHEN WITH CODEINE Robins

Robins

A. H. ROBINS CO., INC., RICHMOND 20, VIRGINIA

Ethical Pharmaceuticals of Merit since 1878

respiratory infections
gastrointestinal infections
genitourinary infections
miscellaneous infections

immediate therapeutic response

use

Sumycin

Squibb Crystalline Tetracycline Phosphate Complex

intramuscular

with Xylocaine*



250 mg. per 1 dose vial
100 mg. per 1 dose vial

- when oral therapy is contraindicated (vomiting, dysphagia, intestinal obstruction, gastrointestinal disorders)
- when the patient is comatose or in shock
- postoperatively

1. fast peak blood and tissue concentrations
2. high cerebrospinal levels
3. for practical purposes, Sumycin is sodium-free

Each vial contains tetracycline phosphate complex equivalent to 250 mg., or 100 mg., of tetracycline HCl. (Note: 250 mg. dose may produce more local discomfort than the 100 mg. dose.)

FLEXIBLE DOSAGE FORMS FOR CONTINUING ORAL THERAPY

	Tetracycline phosphate complex equiv. tetracycline HCl (mg.)	Packaging
Capsules (per capsule)	250	Bottles of 16 and 100
Half Strength Capsules (per capsule)	125	Bottles of 16 and 100
Suspension (per 5 cc. teaspoonful)	125	60 cc. bottles
Pediatric Drops (per cc.—20 drops)	100	10 cc. bottles with dropper

SQUIBB

Squibb Quality—the Priceless Ingredient

*"SUMYCIN" IS A SQUIBB TRADEMARK. ®T.M.® ASTRA PHARMACEUTICAL PRODUCTS, INC.

THE FACTS ON SAFETY WITH

METICORTEN®

prednisone

based on published experience in 4,279 patients

EDEMA.....only 1%

PEPTIC ULCER.....less than 1½%

OSTEOPOROSIS.....only ⅙ of 1%

PSYCHOSIS.....less than ¼ of 1%

rarely seen with METICORTEN– often reported with the more recent steroids — undesirable weight loss; dermatologic side effects

and

never reported with METICORTEN– noted with the more recent steroids — unexplained leg cramps, lightheadedness, headaches, tiredness, weakness, anorexia

1, 2.5 and 5 mg. white tablets

SCHERING CORPORATION · BLOOMFIELD, NEW JERSEY

nothing takes the place of experience especially in long-term steroid therapy

NC-J-1148



TABLET

Cantil® for the colon

plain or
with phenobarbital

"...the most effective available colonic anticholinergic drug."[1]

"...relieves or reduces diarrhea, distention and pain in many patients with functional or organic colon disorders."[2]

EFFECTIVE in ulcerative colitis, irritable colon, mucous colitis, spastic colitis, diverticulitis, diverticulosis, rectospasm, diarrhea following G.I. surgery, bacillary and parasitic disorders.

SELECTIVE activity "...confined principally to the lower gastro-intestinal tract..."[3] and "...singularly free of anticholinergic side-effects...,"[2] such as blurred vision, dry mouth, urinary retention.

CANTIL (plain)—Each scored tablet contains 25 mg. of CANTIL. Bottles of 100 yellow compressed tablets.

CANTIL with Phenobarbital—Each scored tablet contains 25 mg. of CANTIL and 16 mg of phenobarbital (warning: may be habit forming). Bottles of 100 cocoa-brown compressed tablets. CANTIL is the *only* brand of the postganglionic parasympathetic inhibitor N-methyl-3-piperidyl-diphenylglycolate methobromide.

(1) Kleckner, M. S., Jr.: J. Louisiana M. Soc. *108*:359, 1956. (2) Riese, J. A.: Am. J. Gastroenterol. *28*:541, 1957. (3) Kleckner, M. S., Jr.: Clin. Proc. *5*:19, 1957.

LAKESIDE

31259

for pain . . . as effective as codeine without codeine's liabilities

Zactirin*

NON-NARCOTIC

Potently Analgesic

Effectively Anti-inflammatory



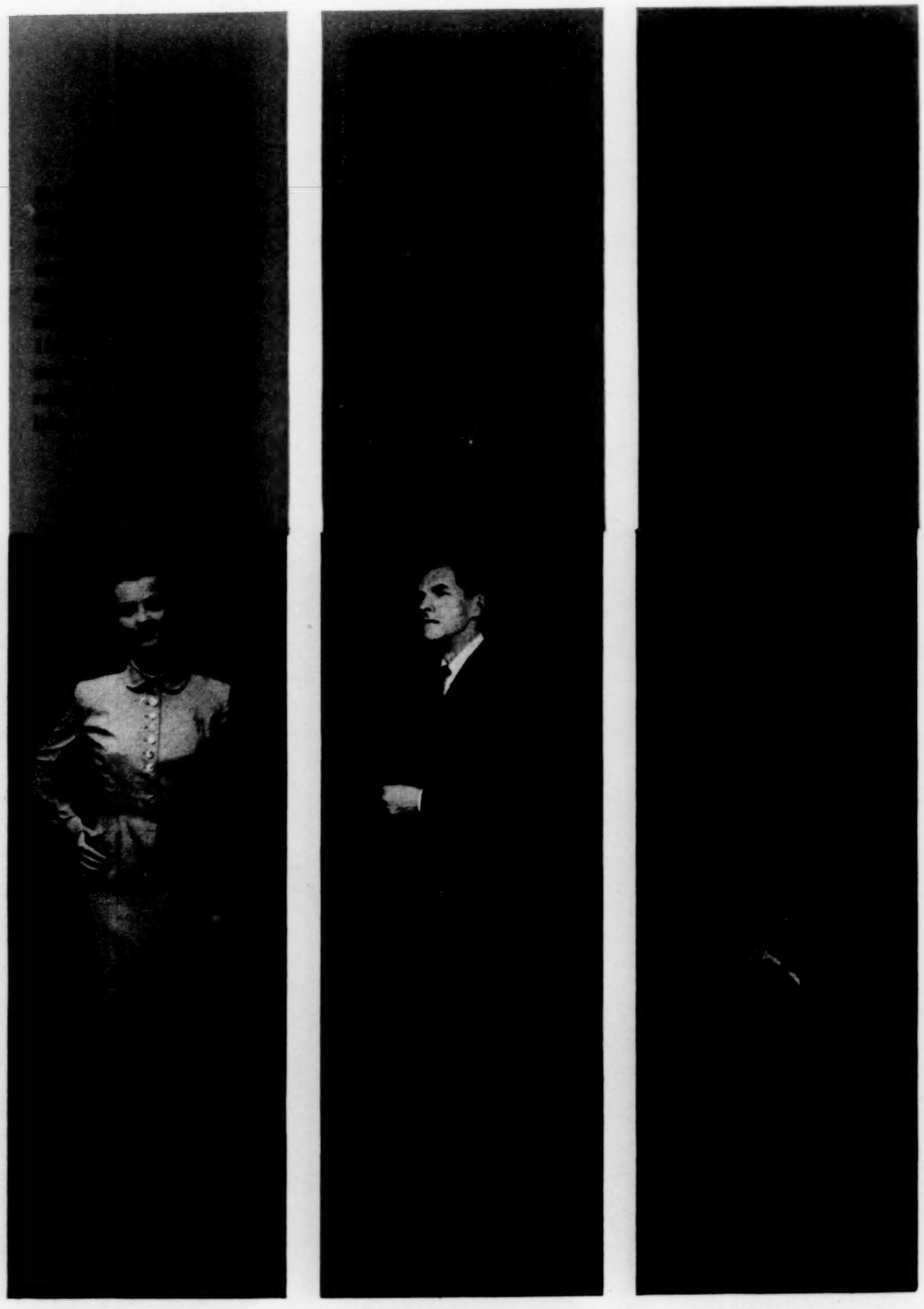
2 ZACTIRIN tablets are equivalent in analgesic potency to ½ grain of codeine plus 10 grains of acetylsalicylic acid.

Supplied: Distinctive, 2-layer yellow-and-green tablets, bottles of 48. Each tablet contains 75 mg. of ethoheptazine citrate and 325 mg. (5 grains) of acetylsalicylic acid.

Wyeth®
Philadelphia 1, Pa.



pattern of subclinical hypothyroidism



pattern for PROLOID®

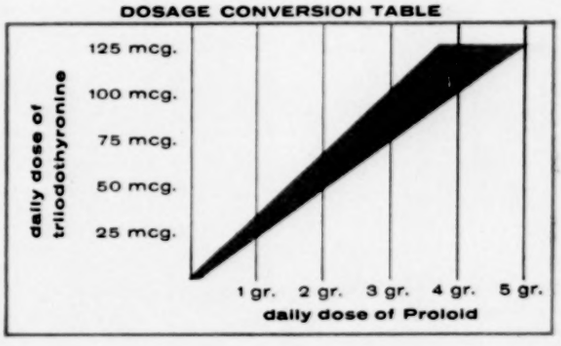
Mild hypothyroidism, a *typical* syndrome is often manifested by *atypical* symptoms. Singly, the complaints of all these patients could be assigned to a variety of conditions. But as you trace the one cause common throughout the group—the typical pattern of mild hypothyroidism emerges.

Often one symptom may supply the clue. A patient's paresthesia, a woman's brittle nails, a child's slow teething may first arouse suspicion. With further investigation, other symptoms fall in line and suspicion may lead to diagnosis.

For final confirmation the physician may turn to the serum PBI test; or, occasionally to empiric confirmation by "break-through" therapy with triiodothyronine. But even when the existence of a hypometabolic state has thus been established (by administration of a synthetic thyroid fraction) long-range thyroid substitution is maintained most successfully and safely with a preparation supplying *all* thyroid fractions in their naturally occurring proportion.

Proloid, the total thyroid complex, fits this specification perfectly. Proloid is standardized both chemically and biologically for metabolic potency. It assures a smooth predictable clinical response.

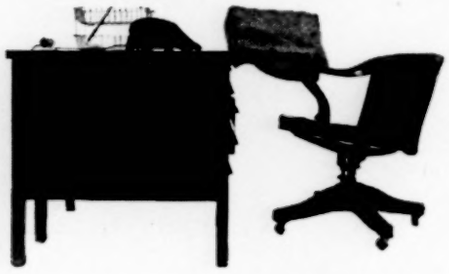
Five Proloid tablet sizes give precision and convenience on every dosage level—for the maintenance of euthyroidism throughout the patient's lifetime. Proloid is prescribed in the same dosage as ordinary thyroid and is available in ¼, ½, 1, 1½ and 5 grain tablets as well as powder.



PROLOID® *the total thyroid complex*

WARNER-CHILCOTT

when you treat common bacterial infections...



a well patient back on the job



measures therapeutic success

Pentids

Squibb 200,000 Unit Buffered Penicillin G Potassium Tablets

when an oral penicillin is indicated...prescribe Pentids

Six years experience by physicians in treating many millions of patients with Pentids confirm clinical effectiveness and safety. Excellent results are obtained with Pentids in many common bacterial infections with only 1 or 2 tablets t.i.d. Pentids may be taken without regard to meals. Pentids are economical . . . cost less than other penicillin salts.

DOSE: 1 or 2 tablets t.i.d. without regard to meals

SUPPLY: Bottles of 12, 100 and 500 tablets

other **Pentids** *products*

NEW Pentids For Syrup: Squibb Flavored Penicillin Powder: when prepared with 35 cc. of water, the preparation provides 60 cc. of fruit-flavored syrup, 200,000 units per teaspoonful (5 cc.).

Pentids Capsules: Squibb Penicillin G Potassium 200,000 Unit Capsules, bottles of 24, 100 and 500.

Pentids Soluble Tablets: Squibb Penicillin G Potassium Soluble Tablets–200,000 units, vials of 12, bottles of 100.

Pentid-Sulfas Tablets: Squibb Penicillin with Triple Sulfas, bottles of 30, 100 and 500.

These formulations are given ½ hr. before meals or 2 hrs. after meals.

SQUIBB *Squibb Quality–the Priceless Ingredient*

"PENTIDS" IS A SQUIBB TRADEMARK

The most effective treatment known for dandruff

ABBOTT LABORATORIES

SELSUN®

(Selenium Sulfide, Abbott)



2 IBEROL Filmtabs a day supply:

THE RIGHT AMOUNT OF IRON

Ferrous Sulfate, U.S.P. ..... 1.05 Gm.
(Elemental Iron—210 mg.)

PLUS THE COMPLETE B COMPLEX

BEVIDORAL® ..... 1 U.S.P. Unit (Oral)
(Vitamin B_{12} with Intrinsic Factor Concentrate, Abbott)

Folic Acid	2 mg.
Liver Fraction 2, N.F.	200 mg.
Thiamine Mononitrate	6 mg.
Riboflavin	6 mg.
Nicotinamide	30 mg.
Pyridoxine Hydrochloride	3 mg.
Calcium Pantothenate	6 mg.

PLUS VITAMIN C

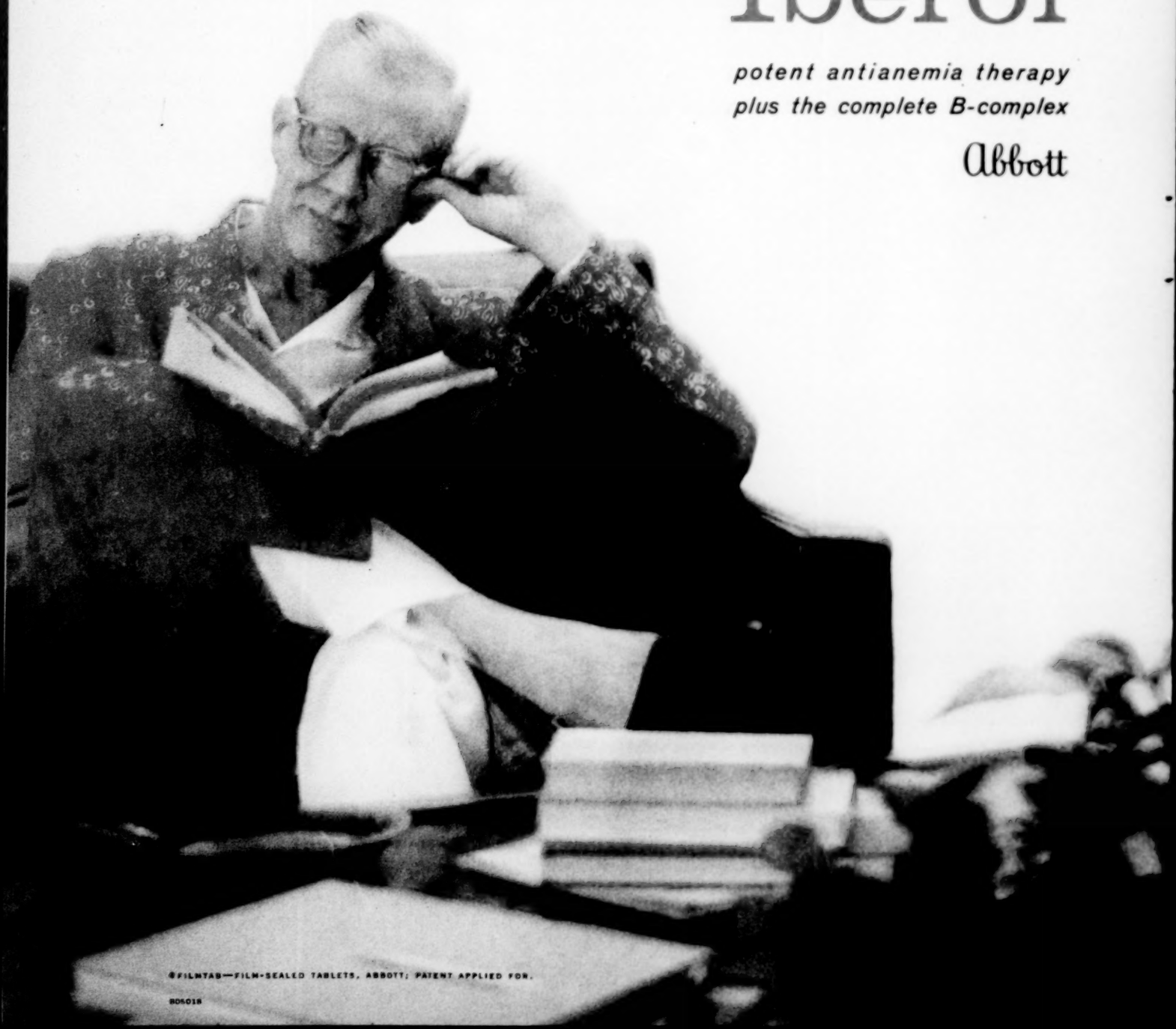
Ascorbic Acid	150 mg.

anemia in convalescence

another indication for filmtab® Iberol®

potent antianemia therapy
plus the complete B-complex

Abbott



®FILMTAB—FILM-SEALED TABLETS, ABBOTT; PATENT APPLIED FOR.

805018

For bacterial diarrheas—new effectiveness . . .

1. Powerful new adsorbent . . .

five times as adsorbent as kaolin

Proof of adsorptive superiority

1 Gm. CLAYSORB plus dye	1 Gm. KAOLIN plus same amount of dye	5 Gm. KAOLIN plus same amount of dye
		
Dye is completely adsorbed.	Much of the dye is still unadsorbed.	Five times as much kaolin is necessary to adsorb the dye.

Supplied: Bottles of 8 fl. oz.

Polymagma®

Wyeth®
Philadelphia 1, Pa.

Dihydrostreptomycin Sulfate, Polymyxin B Sulfate, and Pectin with Claysorb* (Activated Attapulgite, Wyeth) in Alumina Gel, Wyeth

***Trademark*

- POWERFUL NEW ADSORBENT
- SYNERGISTIC ANTIBIOTICS
- LOW DOSE
- PLEASANT TASTE
- WELL-TOLERATED

metaphosphate produced markedly higher blood levels than capsules containing either the corresponding base or the hydrochloride alone. In addition, the average levels derived from the tetracycline base or the chlortetracycline base were higher than those produced by the corresponding hydrochloride though lower than those resulting from the mixture containing the base and sodium metaphosphate. In the study with chlortetracycline[6] capsules containing a mixture of the hydrochloride and sodium metaphosphate were also included in the crossover, and the average levels produced by these capsules were the same as with the mixture of chlortetracycline base with sodium metaphosphate.

Although the enhancement of blood levels of tetracycline by phosphate, either complexed to the tetracycline or mixed with the base or the hydrochloride, thus seemed fairly well established, some doubts still remained because certain reliable observers (including many whose results have not been published) failed to confirm the findings with the materials and methods they used. Further confusion seemed to be added by a subsequent report of Welch et al.,[7] who, in repeating a crossover study with capsules of tetracycline phosphate complex and tetracycline hydrochloride with and without sodium phate, foun[d] much hig[her]

"...Tetracycline hydrochloride and citric acid, in an encapsulated mixture, produced higher serum concentrations and greater urinary excretions, and hence better absorption of tetracyclines, than any other preparation studied..."

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of so[me] were published simultaneously with the last mentioned report of Welch et al.[7] These data were based on thoroughly controlled studies both in rats[8] and in man[9] and include additional findings that serve to explain, fairly conclusively, the various discrepancies that have been mentioned.

The experiments in rats[8] were carried out to study the effects of citric acid, dicalcium phosphate, sodium metaphosphate, food, oil and sorbitol on the serum antibacterial activity produced by the administration of tetracycline hydrochloride or tetracycline base. Citric acid administered in equal weight with tetracycline hydrochloride gave the highest concentrations of all the preparations studied. No enhancing effect was obtained from citric acid when given with tetracycline base. Dicalcium phosphate and food resulted in lower, and sodium metaphosphate in higher, serum antibacterial activity than was observed in their absence. Oil and sorbitol did not interfere with tetracycline absorption.

Dicalcium phosphate is widely used as a filler in various capsules, including those of the tetracyclines. The authors cite a large number of other studies that implicate the presence of calcium ions as the cause of the reduced absorption of tetracyclines and show that citric acid can partially neutralize this effect. The depressing effect of food on the serum levels of tetracycline is likewise explained by the goodly amount of minerals contained in commercial laboratory diets, and they postulate that the multivalent cations may be responsible for the poorer absorption of the drug. The authors could not explain the failure of citric acid to enhance serum concentrations when administered with tetracycline base in contrast to its marked effect when given as the hydrochloride. However, they hypothesized that the ability of citric acid to enhance serum levels of tetra[cycl]ine is re[lated] to its ability to form complexes [which] are unavailable for [absorption].

paper of
et al.[7] indicates that in their study the capsules
tetracycline hydrochloride, chlortetracycline hydrochloride and tetracycline phosphate complex all contained dicalcium phosphate as a filler, whereas the capsules containing citric acid and sodium hexametaphosphate did not contain any dicalcium phosphate. This could clearly explain the discrepancies noted in that study. Likewise, the inconsistencies in other studies may very well have been due to the presence of calcium as fillers in some of the capsules and not in others.

This, however, fails to explain the most recent findings of Welch and Wright,[10] who compared the absorption of three capsules, each containing 250 mg. of oxytetracycline hydrochloride — one without any adjuvant, one with 250 mg. of citric acid and the third with 380 mg. of sodium hexametaphosphate; no other filler was contained in any of these capsules. In triple

crossover studies, they as
lected one, three and six
found that sodium hexa
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Enhancement of serum le
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Tobacco and Health,
Looks at Smoking, di

Editorial.
The New England Journal of Medicine.
258:97-99, (January 9) 1958

ACHROMYCIN* V

TETRACYCLINE HCl BUFFERED WITH CITRIC ACID

is

tetracycline and citric acid

Lederle

LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY, PEARL RIVER, NEW YORK

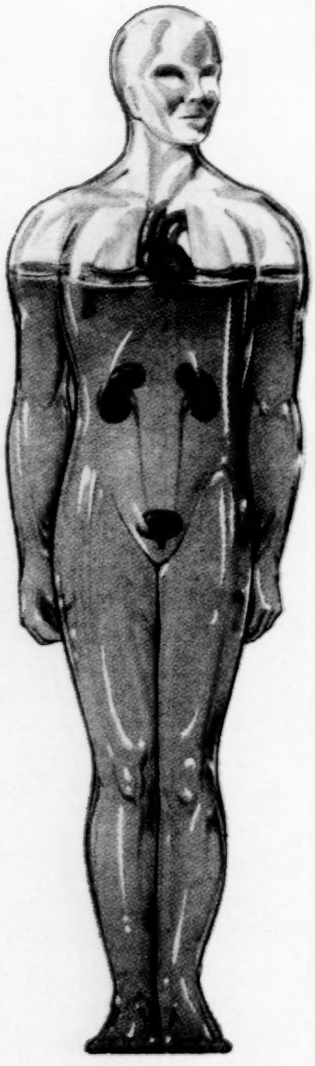
*Reg. U. S. Pat. Off.

'DIURIL'

(CHLOROTHIAZIDE)

in

EDEMA



Start therapy with one or two 500 mg. tablets of 'DIURIL' once or twice a day.

BENEFITS:

- The only orally effective nonmercurial agent with diuretic activity equivalent to that of the parenteral mercurials.
- Excellent for initiating diuresis and maintaining the edema-free state for prolonged periods.
- Promotes balanced excretion of sodium and chloride—without acidosis.

Any indication for diuresis is an indication for 'DIURIL':

Congestive heart failure of all degrees of severity; premenstrual syndrome (edema); edema and toxemia of pregnancy; renal edema—nephrosis; nephritis; cirrhosis with ascites; drug-induced edema. May be of value to relieve fluid retention complicating obesity.

SUPPLIED: 250 mg. and 500 mg. scored tablets 'DIURIL' (chlorothiazide); bottles of 100 and 1,000.

'DIURIL' and 'INVERSINE' are trade-marks of Merck & Co., Inc.

MSD MERCK SHARP & DOHME
Division of MERCK & CO., INC., Philadelphia 1, Pa.

as simple
as 1-2-3
in

HYPERTENSION

1 INITIATE 'DIURIL' THERAPY

'DIURIL' is given in a dosage range of from 250 mg. twice a day to 500 mg. three times a day.

2 ADJUST DOSAGE OF OTHER AGENTS

The dosage of other antihypertensive medication (reserpine, veratrum, hydralazine, etc.) is adjusted as indicated by patient response. If the patient is established on a ganglionic blocking agent (e.g., 'INVERSINE') this should be continued, but the total daily dose should be *immediately* reduced by 25 to 50 per cent. This will reduce the serious side effects often observed with ganglionic blockade.

3 ADJUST DOSAGE OF ALL MEDICATION

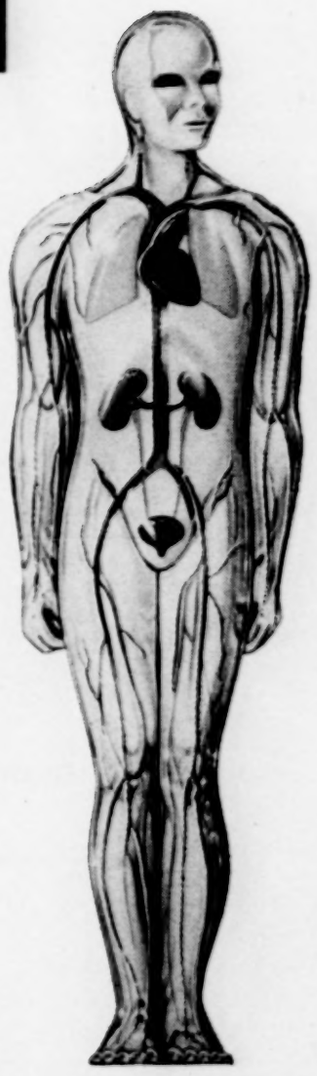
The patient must be frequently observed and careful adjustment of all agents should be made to determine optimal maintenance dosage.

BENEFITS:

- **improves and simplifies the management of hypertension**
- **markedly enhances the effects of antihypertensive agents**
- **reduces dosage requirements for other antihypertensive agents—often below the level of distressing side effects**
- **smooths out blood pressure fluctuations**

INDICATIONS: management of hypertension

Smooth, more trouble-free management of hypertension with 'DIURIL'



Keep a tube of TASHAN® handy—



It works wonders in
dry, scaly skin, chafing, chapping,
detergent rash, diaper rash,
intertrigo, simple eczema, minor
burns, excoriation, superficial
ulcers and fissures.

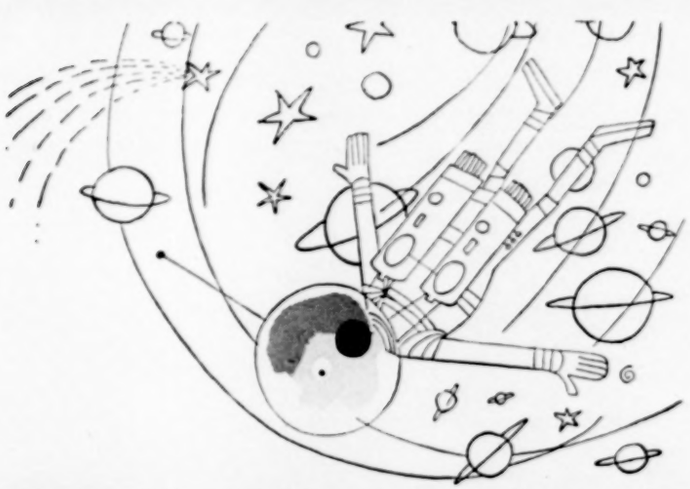
ANTIPRURIENT · SOOTHING · HEALING

Contains vitamins A, D, E,
and *d*-panthenol in a
water-miscible, non-sensitizing base.

Roche—Reg. U. S. Pat Off.

ROCHE LABORATORIES
Division of Hoffmann-La Roche Inc
Nutley, New Jersey

ℓ-GLUTAVITE



BRING HIM BACK FROM OUTER SPACE
to feed the inner man



With REDISOL (vitamin B_{12})—new zest for meals. Soluble REDISOL tablets (25, 50, 100, 250 mcg.) and cherry-flavored REDISOL Elixir (5 mcg. per 5 cc.) mix readily with liquids.

REDISOL is a trade-mark of Merck & Co., Inc.

MSD **MERCK SHARP & DOHME**
DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

PROVED ANTIBIOTIC DIFFUSION

Tetrex®
Tetracycline Phosphate Complex **CAPSULES**

PROVIDES
HIGH
TETRACYCLINE
CONCENTRATIONS
IN BODY FLUIDS
AND TISSUES

portal vein
4.8 mcg./ml.[2]
(at 2 hrs.
after 500 mg. dose)

bile
33.5 mcg./ml.[1]
(max. av.—6 hrs.
after 2nd 500 mg. dose)

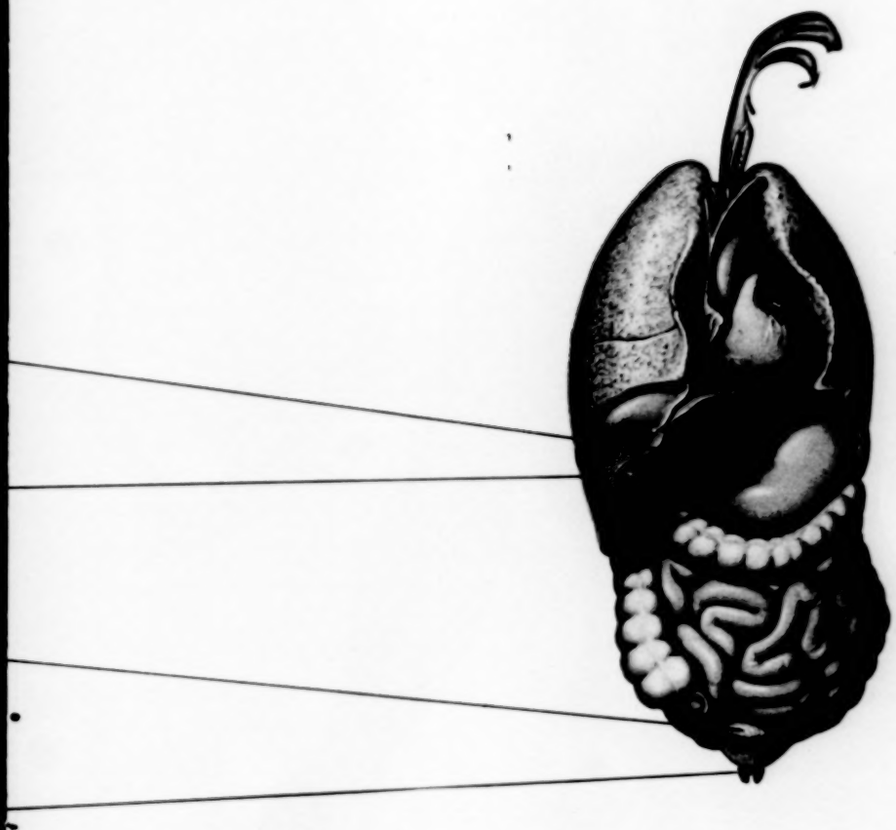
urine
50.3 mcg./ml.[3]
(av. concentration
following 500 mg. dose)

prostatic fluid
1.77 mcg./ml.[1]
(av. at 2 hrs.
after 2nd 500 mg. dose)

FOR INTENSIFIED BROAD-SPECTRUM ANTIBIOTIC CONTROL

1. Pulaski, E. J., and Isokane, R. K.: Antibiotic Med. 4:408, 1957.
2. Shidlovsky, B. A., et al.: In Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 459.
3. Buckwalter, F. H., and Cronk, G. A.: Antibiotic Med. 5:46, 1958.

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LABORATORIES INC.
SYRACUSE, NEW YORK

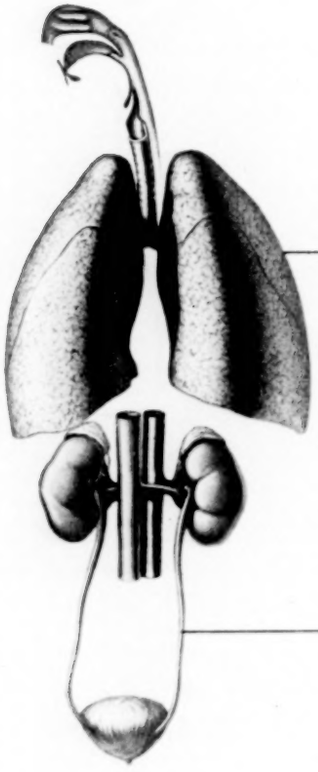


Tetrex®

Tetracycline Phosphate Complex **250 • 100 mg. CAPSULES**

"DUANAT" REPRODUCTION, PAT. PENDING.

Bristol
LABORATORIES INC.
SYRACUSE, NEW YORK



Tetrex®

Tetracycline Phosphate Complex 250 • 100 mg. CAPSULES

"DUANAT" REPRODUCTION, PAT. PENDING.

ACTION AT THE POINT OF INFECTION

in 232 respiratory infections:
"All patients infected with tetracycline-sensitive organisms responded satisfactorily . . ."[1]

in 94 dermatologic infections
"88 per cent . . . obtained excellent or good improvement . . ."[2]

in 30 g.u. infections:
effective in all cases of urethritis, cystitis, pyuria.[1,3]

in 103 soft-tissue infections:
"marked decrease in morbidity and hospitalization" and "incidence and magnitude of the [surgical] procedures was lessened."[4]

Tetrex®

Tetracycline Phosphate Complex **CAPSULES**
U.S. Pat. 2,791,609

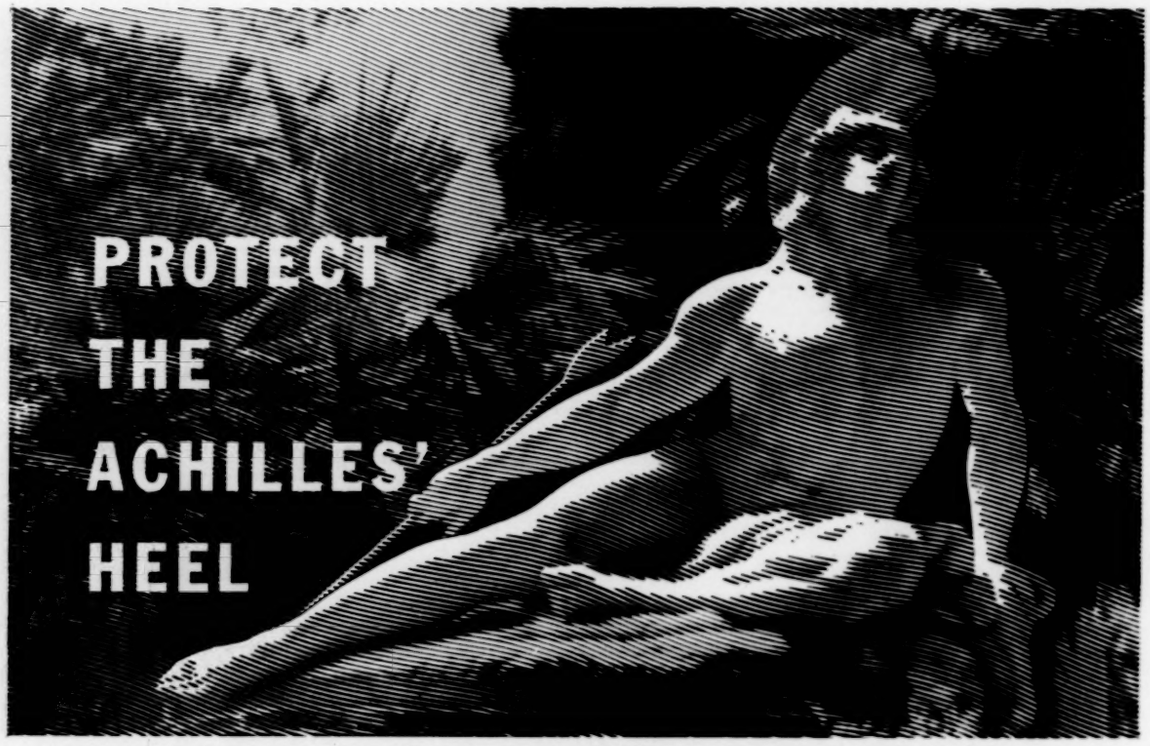
For antibiotic action at the foci of infection, TETREX provides rapid, uniformly successful control of tetracycline-sensitive organisms...as demonstrated by the results in 826 systemic and local infections[1-4] (including those illustrated).

A pure antibiotic compound, not a mixture —"more rapidly and completely absorbed . . ."[5] than tetracycline HCl, providing fast, high concentrations in body fluids and tissues.

Highly flexible in dosage . . . equally effective on b.i.d. (500 mg.) or q.i.d. (250 mg.) schedule[1-3] with equally excellent toleration.

SUPPLIED: TETREX Capsules containing the equivalent of 250 mg. tetracycline HCl activity; bottles of 16 and 100. *New* TETREX Pediatric Capsules containing the equivalent of 100 mg. tetracycline HCl activity; bottles of 25 and 100.

1. Cronk, G. A., et al.: In Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 397. 2. Rein, C. R., and Fleischmajer, R.: Antibiotic Med. 4:422, 1957. 3. Putnam, L. E.: Ibid. 4:470, 1957. 4. Prigot, A., et al.: Ibid. 4:287, 1957. 5. Council on Drugs, A.M.A.: J.A.M.A. 166:50, 1958.



OF EVERY AGING PATIENT

The combination of declining gonadal function and increased vulnerability to malnutrition conspire to accelerate the aging process. You can protect the aging patient by prescribing a "Clusivol" Geriatric capsule daily.

There are four important features of "Clusivol" Geriatric:

1. **Vitamins** – 12 important nutritional supplements, notably vitamins A and D, the factors of the B complex, and vitamin C.
2. **Minerals and trace elements** – 10 protective factors to ensure optimal blood and bone building.
3. **Amino acids** – lysine and methionine, key amino acids usually lacking in finicky geriatric diets.
4. **Gonadal steroids** – estrogen and androgen in small quantities to restore the integrity of the body mechanism.

Supplied: No. 294 – Capsules, bottles of 100 and 1,000.

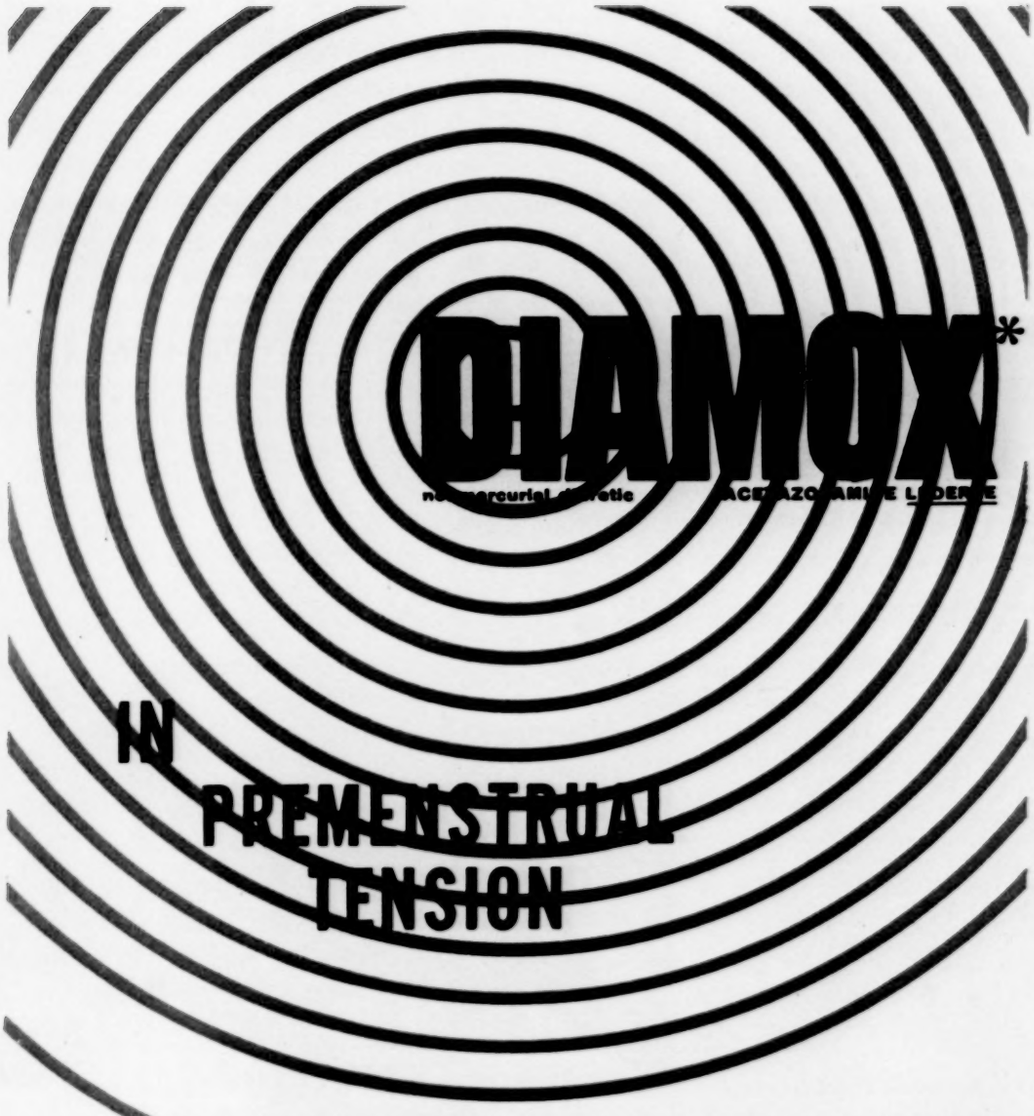
"CLUSIVOL"® GERIATRIC

potent nutritional elements with steroids

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AN EVER-WIDENING CIRCLE OF THERAPEUTIC INFLUENCE

Welcome relief can be the rule rather than the exception in the treatment of premenstrual tension so often associated with fluid retention.

Patients report marked improvement of physical and emotional well-being on a simple regimen of DIAMOX—½–1½ tablets daily, depending on weight. Treatment begins 6–10 days prior to onset of menstruation, or at the onset of symptoms.

Well-tolerated, a single oral dose of DIAMOX offers convenient daytime diuresis and nighttime rest.

Supplied: Scored tablets of 250 mg.; Syrup containing 250 mg. per 5 cc. teaspoonful and vials of 500 mg. for parenteral use.

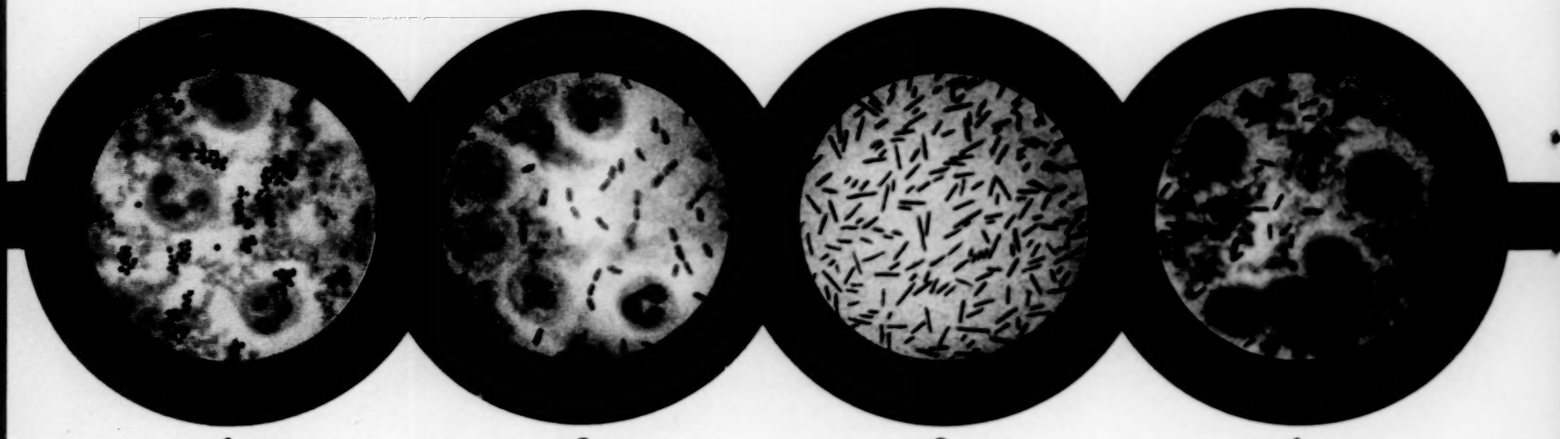
LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY, PEARL RIVER, NEW YORK Lederle
*Reg. U.S. Pat. Off.

THIS 5-YEAR STUDY SHOWS... CONTINUED EFFICACY

CHLOROMYCETIN®

COMBATS MOST CLINICALLY IMPORTANT PATHOGENS

Recent reports comparing the effectiveness of various antibiotics against commonly encountered pathogens indicate that CHLOROMYCETIN (chloramphenicol, Parke-Davis) has maintained its high degree of effectiveness.[1-5] It is still highly active against many strains of staphylococci,[1-8] streptococci,[2,7] pneumococci,[2] and gram-negative[1,2,7,9,10] organisms.



1 2 3 4

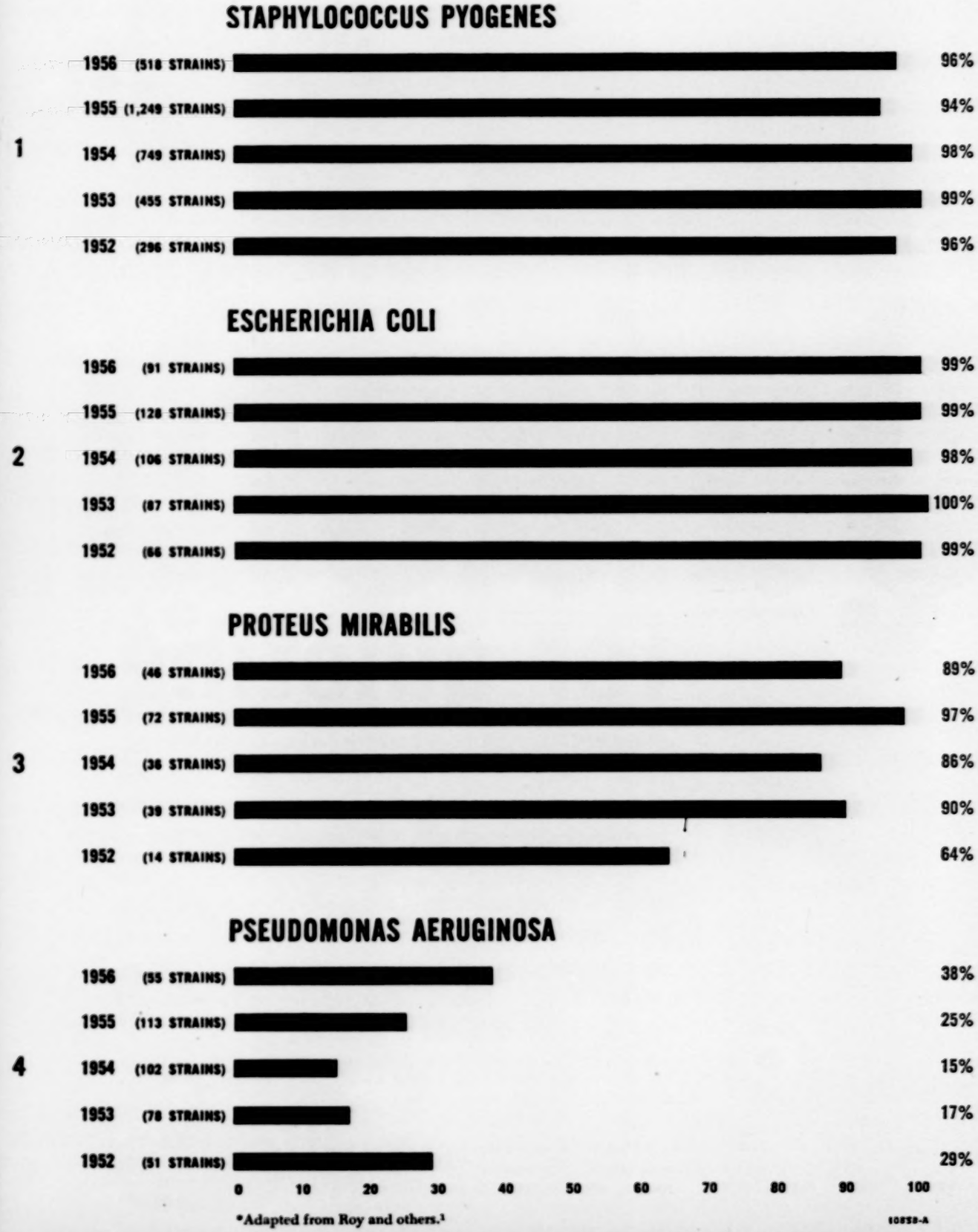
CHLOROMYCETIN is a potent therapeutic agent, and because certain blood dyscrasias have been associated with its administration, it should not be used indiscriminately or for minor infections. Furthermore, as with certain other drugs, adequate blood studies should be made when the patient requires prolonged or intermittent therapy.

REFERENCES: (1) Roy, T. E.; Collins, A. M.; Craig, G., & Duncan, I. B. R.: *Canad. M.A.J.* 77:844 (Nov. 1) 1957. (2) Schneierson, S. S. *J. Mount Sinai Hosp.* 25:52 (Jan.-Feb.) 1958. (3) Koch, R., & Donnell, G.: *California Med.* 87:313, 1957. (4) Waisbren, B. A., & Strelitzer, C. L.: A Five-Year Study of the Antibiotic Sensitivities and Cross Resistances of Staphylococci in a General Hospital, paper presented at Fifth Ann. Symp. on Antibiotics, Washington, D. C., Oct. 2-4, 1957. (5) Doniger, D. E., & Parenteau, Sr. C. M.: *J. Maine M. A.* 48:120, 1957. (6) Royer, A.: Changes in Resistance to Various Antibiotics of Staphylococci and Other Microbes, paper presented at Fifth Ann. Symp. on Antibiotics, Washington, D. C., Oct. 2-4, 1957. (7) Hasenclever, H. F.: *J. Iowa M. Soc.* 47:136, 1957. (8) Josephson, J. E., & Butler, R. W.: *Canad. M.A.J.* 77:567 (Sept. 15) 1957. (9) Rhoads, P. S.: *Postgrad. Med.* 21:563, 1957. (10) Holloway, W. J., & Scott, E. G.: *Delaware M. J.* 29:159, 1957.

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MEDICAMENTA VERA · PD CO.

IN VITRO SENSITIVITY OF FOUR COMMON PATHOGENS TO CHLOROMYCETIN FROM 1952 TO 1956*



*Adapted from Roy and others.[1]

10858-A

FINALLY! A TRANQUILIZER THAT WILL stop SHORT OF DROWSINESS

Quiactin

(oxanamide)

for quieting

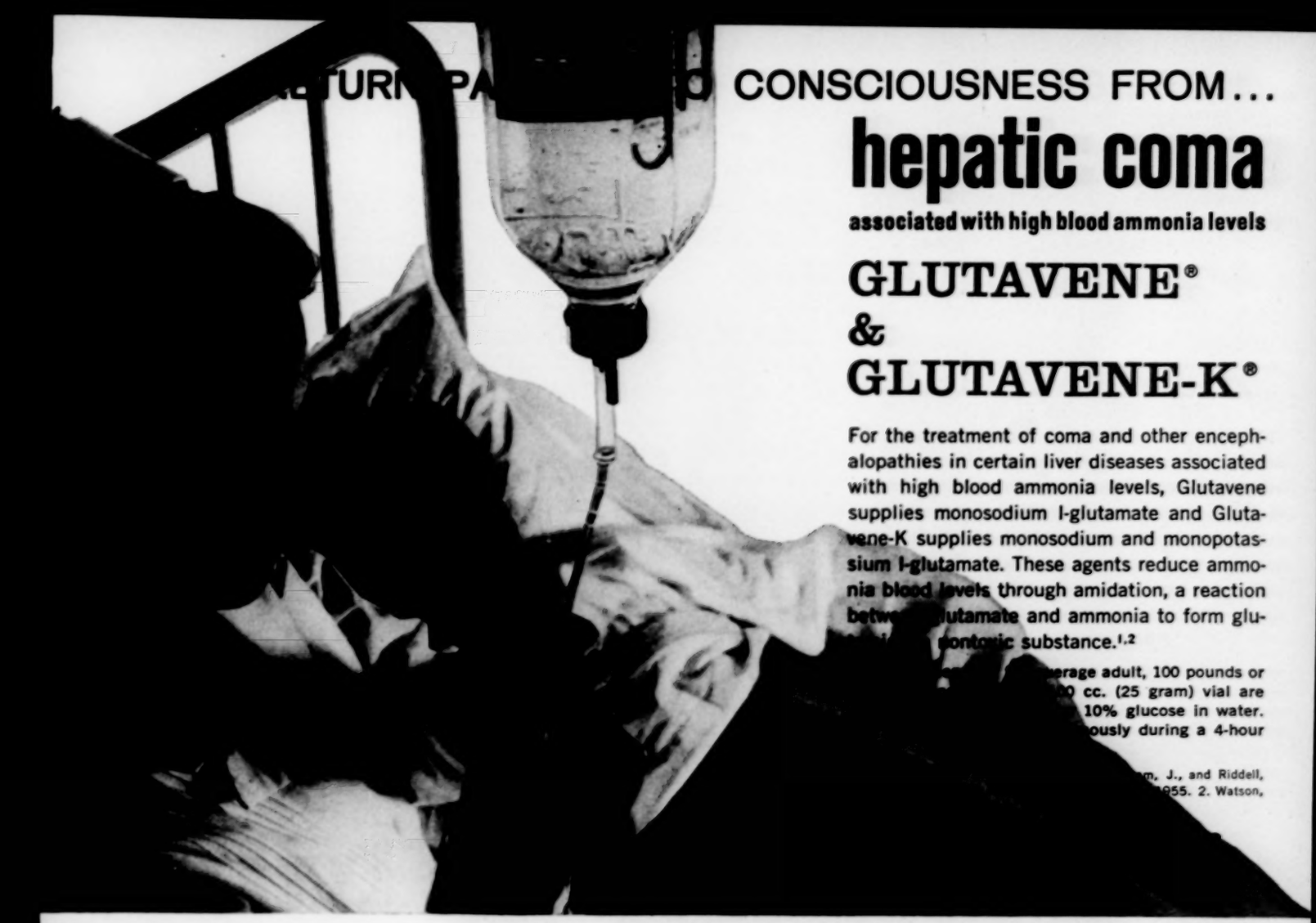
QUIACTIN—in the recommended dose—one 400 mg. tablet q.i.d., provides greater tranquility with less drowsiness and more prolonged activity.[1] QUIACTIN is remarkably nontoxic, noncumulative and has no withdrawal symptoms.[1-3]

Structurally, QUIACTIN is a completely new tranquilizer... therapeutically, it's different... stops before it goes farther than patient comfort or safety allows. QUIACTIN does not push the patient beyond tranquility into lassitude, dullness, depression.

1. Proctor, R. C.: Dis. Nerv. Sys. 18:223 1957. 2. Feuss, C. D., and Gragg, L., Jr.: Dis. Nerv. Sys. 18:29, 1957. 3. Coats, E. A., and Gray, R. W.: Dis. Nerv. Sys. 18:191, 1957. Registered Trademark: Quiactin

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CONSCIOUSNESS FROM...

hepatic coma

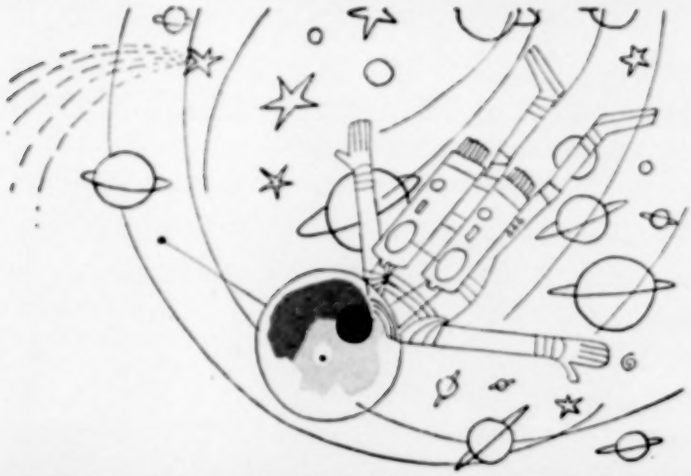
associated with high blood ammonia levels

GLUTAVENE®
&
GLUTAVENE-K®

For the treatment of coma and other encephalopathies in certain liver diseases associated with high blood ammonia levels, Glutavene supplies monosodium l-glutamate and Glutavene-K supplies monosodium and monopotassium l-glutamate. These agents reduce ammonia blood levels through amidation, a reaction between glutamate and ammonia to form glutamine, a nontoxic substance.[1,2]

...erage adult, 100 pounds or ...0 cc. (25 gram) vial are ... 10% glucose in water. ...ously during a 4-hour

...m, J., and Riddell, ...955. 2. Watson,



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to feed the inner man



With REDISOL (vitamin B_{12})—new zest for meals. Soluble REDISOL tablets (25, 50, 100, 250 mcg.) and cherry-flavored REDISOL Elixir (5 mcg. per 5 cc.) mix readily with liquids.

REDISOL is a trade-mark of Merck & Co., Inc.

MSD **MERCK SHARP & DOHME**
DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

you can determine gastric acidity simply, effectively and without intubation, use new



Diagnex Blue

Squibb Azure A Carbacrylic Resin Diagnostic Test

. . . the visual color test for gastric acidity

How to Use

1. The patient takes Diagnex Blue orally.
2. Urine samples are returned to the physician.
3. Simple color comparison denotes gastric acid status.

Advantages

- a simplified, visual method without intubation
- highly dependable results
- time-saving convenience—can be used right in the office
- no fluorescent light or other special equipment needed

Indications

The Diagnex Blue test is a simple, highly useful step in the diagnosis of conditions associated with abnormal gastric acid secretion. According to Goldbloom and his associates, "the test for achlorhydria is extremely valuable in the diagnosis of pernicious anemia, cancer of the stomach, and gastric polyps."

Boxes of 5 and 50 Diagnex Blue test units with comparators.

Goldbloom, A. A., Feinstein, M. A. and Eiber, H. B.: *Am. J. Dig. Dis.*, *22*:288, 1955.

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Squibb Quality—the Priceless Ingredient

"DIAGNEX" BLUE IS A SQUIBB TRADEMARK



"This substance [Vitamin K_1] has added greatly to the safety of anticoagulant therapy"[1]

reverse anticoagulant-induced hypoprothrombinemia

MEPHYTON®

VITAMIN K_1

the only available preparation chemically identical with naturally-occurring vitamin K_1... "has a more prompt, more potent and more prolonged effect than the vitamin K analogues"[2]

Dosage: Orally, to modify anticoagulant effects: 5 to 10 mg. initially; 15 to 25 mg. for more vigorous action. Intravenously, for anticoagulant-induced bleeding emergencies, 10 to 50 mg.; may be repeated as indicated by prothrombin time response. (Some clinicians advise their patients to keep a supply of tablets on hand at all times; if gross bleeding occurs, the patients are instructed to take 10 mg. and phone the doctor.[1])

Supplied: Tablets, 5 mg., bottles of 100. Emulsion, each 1-cc. ampul contains 50 mg., boxes of 6 ampuls.

Other indications: To normalize prothrombin time—before surgery, in obstructive jaundice, hepatic disease, impaired gastrointestinal absorption, deficiency of vitamin K in the newborn, and following the administration of antibiotics, sulfonamides, and salicylates. 'Mephyton' is a valuable addition to the physician's bag for emergency use.

MERCK SHARP & DOHME
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Mephyton is a trade-mark of MERCK & CO., INC.

1. Wright, I. S.: Early use of anticoagulants in treatment of myocardial infarction, J.A.M.A. 163: 918-921, March 16, 1957.
2. Council on Pharmacy and Chemistry: New and Nonofficial Remedies, Philadelphia, J. B. Lippincott Co., 1956, p. 505.

EMDEE* MARGARINE

a delicious table spread

a versatile kitchen shortening



its high corn oil content will not elevate blood cholesterol levels

In Emdee Margarine, 80% of the fat content consists of nonhydrogenated corn oil which has been specially processed to preserve its original content of unsaturated fatty acids. Each 100 Gm. provides 34 Gm. of linoleic acid and 18 Gm. of other unsaturated fatty acids.

Daily use of Emdee Margarine instead of customary table spreads and cooking fats puts a safety factor in the diet... assures patient acceptance... provides a liberal intake of the *unsaturated* fatty acids believed essential in metabolism and important in control of blood cholesterol levels.

Supplied in 1 pound cans, to be kept refrigerated when not in use.

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UNIQUE Robin- *research discovery*

for SELECTIVE, SUPERIOR

skeletal muscle relaxation



Robaxin

Supply

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HYPERTENSIVE...yet controlled
with safer combination therapy

"objective relief...gratifying"

Rauvera—the combination of alseroxylon and alkavervir—is much more effective than either drug alone. This combination produces "no postural hypotension, no organ toxicity, and no sensitization reactions. Tolerance does not develop on prolonged administration...hypotensive action is steady and prolonged and persists over the entire twenty-four hours.[1]" Rauvera therapy can be continued over long periods of time.

"subjective relief...even more so"[1]

Alseroxylon and alkavervir "when combined produce mutual reinforcement so that...more severe cases respond," yet "side effects are minimal."[2] Most patients feel better, are less tired and are free from headaches.[3] Anxiety and tension are relieved...pulse rate slowed...such symptoms as "heart consciousness," tinnitus, vertigo, giddiness and insomnia disappear rapidly —leaving a calm and relaxed patient.

RAUVERA®

Each scored tablet of Rauvera contains 1 mg. purified Rauwolfia serpentina alkaloids (alseroxylon) and 3 mg. alkavervir, biologically standardized.

1. Bendig, A.: New York State J. M. 66:2523, 1956. 2. La Barbera, J. F.: Med. Rec. and Ann. 50:242, 1956. 3. Gilchrist, A. R.: Brit. M. J. No. II:1011, 1956.

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complete vitamin-mineral supplementation for pregnancy and lactation, with these important extras...

- new, better tolerated source of iron
- noninhibitory intrinsic factor to augment B_{12} absorption
- important trace elements
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So she won't forget FILIBON...

- the FILIBON Jar, fashioned for her, will keep her on the regimen you prescribe
- FILIBON capsules are small, easy to swallow
- the FILIBON dosage is convenient—only one a day

Each soft-shell FILIBON capsule contains:

Vitamin A	4,000 U.S.P. Units	Ferrous Fumarate	90 mg.
Vitamin D	400 U.S.P. Units	Iron (as Fumarate)	30 mg.
Thiamine Mononitrate (B_1)	3 mg.	Intrinsic Factor	5 mg.
Pyridoxine (B_6)	1 mg.	Fluorine (CaF_2)	0.015 mg.
Niacinamide	10 mg.	Copper (CuO)	0.15 mg.
Riboflavin (B_2)	2 mg.	Iodine (KI)	0.01 mg.
Vitamin B_{12}	2 mcgm.	Potassium (K_2SO_4)	0.835 mg.
Ascorbic Acid (C)	50 mg.	Manganese (MnO_2)	0.05 mg.
Vitamin K (Menadione)	0.5 mg.	Magnesium (MgO)	0.15 mg.
Folic Acid	1 mg.	Molybdenum ($Na_2MoO_4 . 2H_2O$)	0.025 mg.
		Zinc (ZnO)	0.085 mg.
		Calcium Carbonate	575 mg.

in the picture...during pregnancy

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DOSAGE / one or more capsules daily

SUPPLIED / attractive re-usable bottles of 100 capsules

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new, lifesaving Furadantin®

brand of nitrofurantoin

intravenous solution for severe infections



A WIDE RANGE OF CLINICAL USEFULNESS...

in systemic infections such as septicemia (bacteremia), peritonitis, and other bacterial infections as of postoperative wounds and abscesses. *In severe genitourinary tract infections* when the patient is unable to take FURADANTIN per os.

...AND A WIDE RANGE OF BACTERICIDAL EFFECTIVENESS...

wide-spectrum activity against most common pathogens • clinically effective against many antibiotic- or sulfonamide-resistant genera such as Aerobacter, Staphylococcus, Proteus, and certain strains of Pseudomonas.

...WITH CERTAIN UNIQUE ADVANTAGES

• negligible development of bacterial resistance • no reports of renal, hematopoietic or hepatic toxicity • no monilial superinfection • safe for continuous use without danger of thrombophlebitis

Full dosage instructions and discussion of indications and side effects are enclosed in each package. FURADANTIN *Intravenous Solution* is available to all hospital pharmacies.

NITROFURANS—a *new* class of antimicrobials— neither antibiotics nor sulfonamides

EATON LABORATORIES, NORWICH, NEW YORK

An added benefit for hypertensive patients

CIBA antihypertensive agents reduce cholesterol and lipoprotein levels

Reports of the cholesterol- and lipoprotein-reducing properties of Serpasil, Apresoline and the ganglionic blockers have come from several groups of investigators. Corcoran *et al.*[1] noted that atherosclerotic complications were less common in those with good responses to antihypertensive treatment than those with fair or poor response. Under Apresoline therapy, the average depression of cholesterol in 112 unselected hypertensive patients was 29 mg.; ". . . among the 66 with high control levels the fall was 48.2 mg. in the first six months, and thereafter 43.5 mg. per 100 ml. of plasma."[2] Within a group of 9 geriatric patients given reserpine, ". . . seven patients showed a definite drop in the blood level of the heavier and more compact lipoprotein molecules . . ."[3]

When planning a program to reduce blood-pressure levels, consider the added benefits of these CIBA antihypertensive agents:

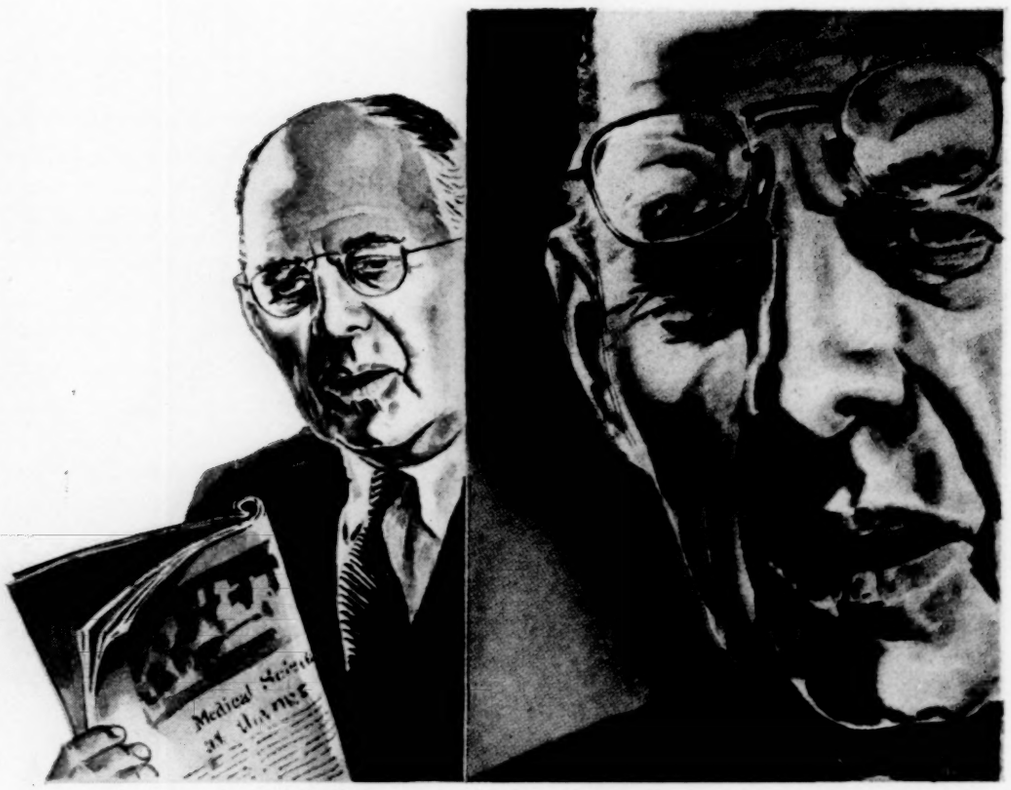
Serpasil®... Apresoline®

1. Corcoran, A. C., Lewis, L. A., Dustan, H. P., and Page, I. H.: Ann. New York Acad. Sc. *64*:620 (Nov. 16) 1956. 2. Perry, H. M., Jr., and Schroeder, H. A.: J. Chronic Dis. *2*:520 (Nov.) 1955. 3. Galioni, E. F.: California Med. *85*:97 (Aug.) 1956.

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Physicians who have used PROTAMIDE extensively deplore such statements as unfortunate when they appear in the lay press. They have repeatedly observed in their practice quick relief of pain, even in severe cases, shortened duration of lesions, and greatly lowered incidence of postherpetic neuralgia when PROTAMIDE was started promptly. A folio of reprints is available. These papers report on zoster in the elderly—the severely painful cases—patients with extensive lesions. PROTAMIDE users know "shingles" *can* be helped.



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Available: Boxes of 10 ampuls—prescription pharmacies.

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HIGH POTENCY CORTICOID CONTROL

whenever oral therapy is impracticable











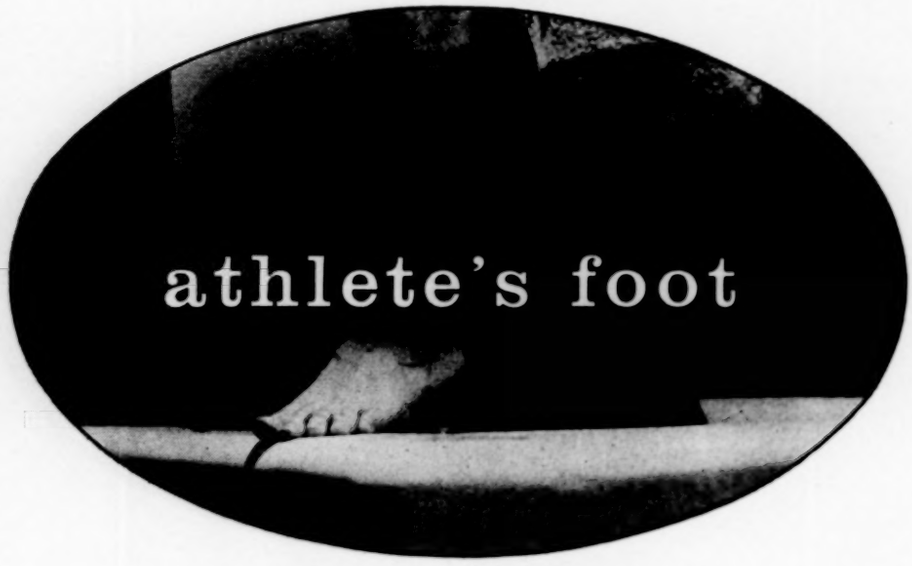
in high dosage therapy
in patients undergoing surgery
in the severely ill or debilitated
in presence of vomiting, coma
in the presence of shock

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the first high potency corticoid designed specifically for intramuscular use

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carrier unto himself

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fast relief from itching

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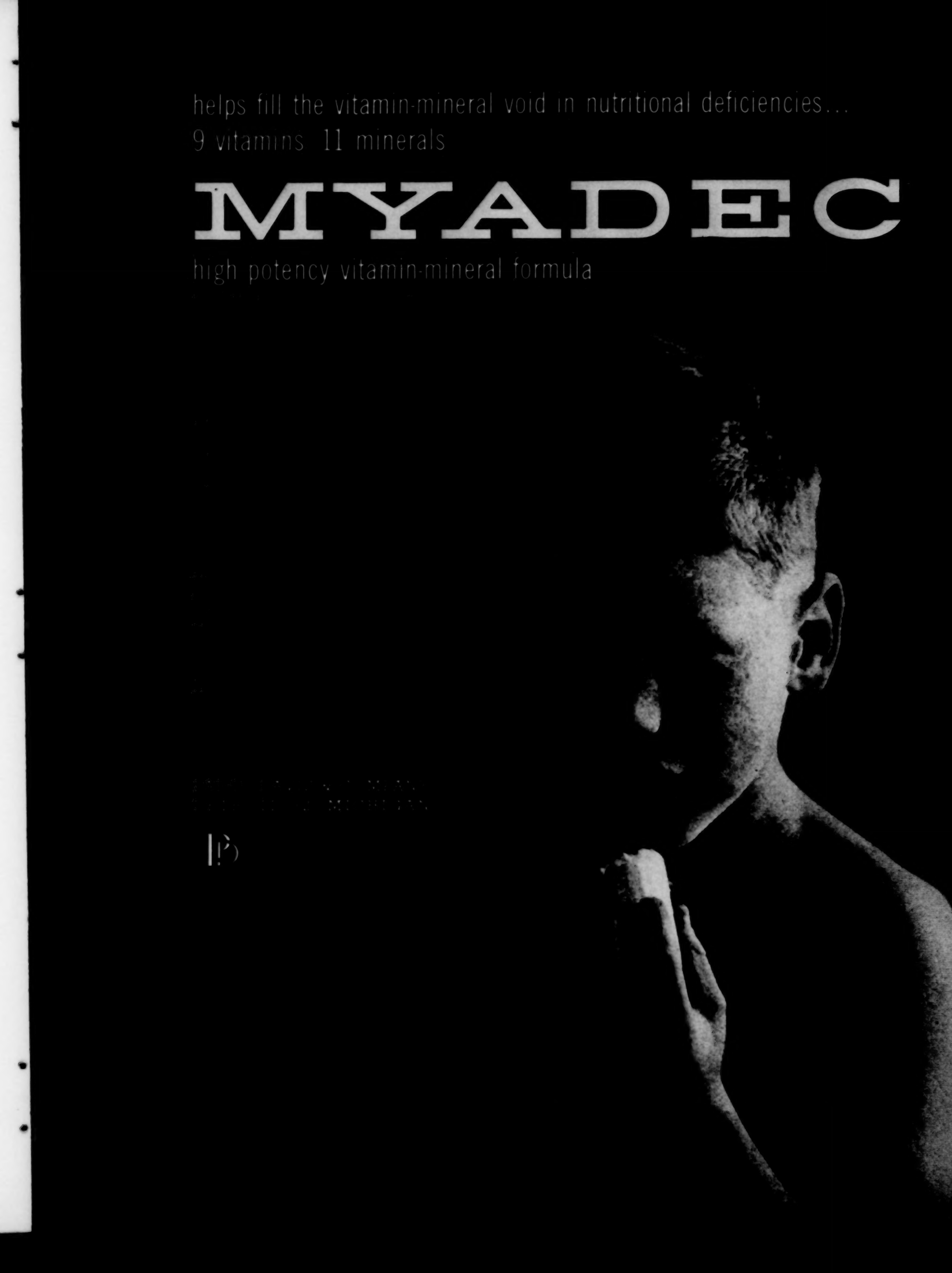
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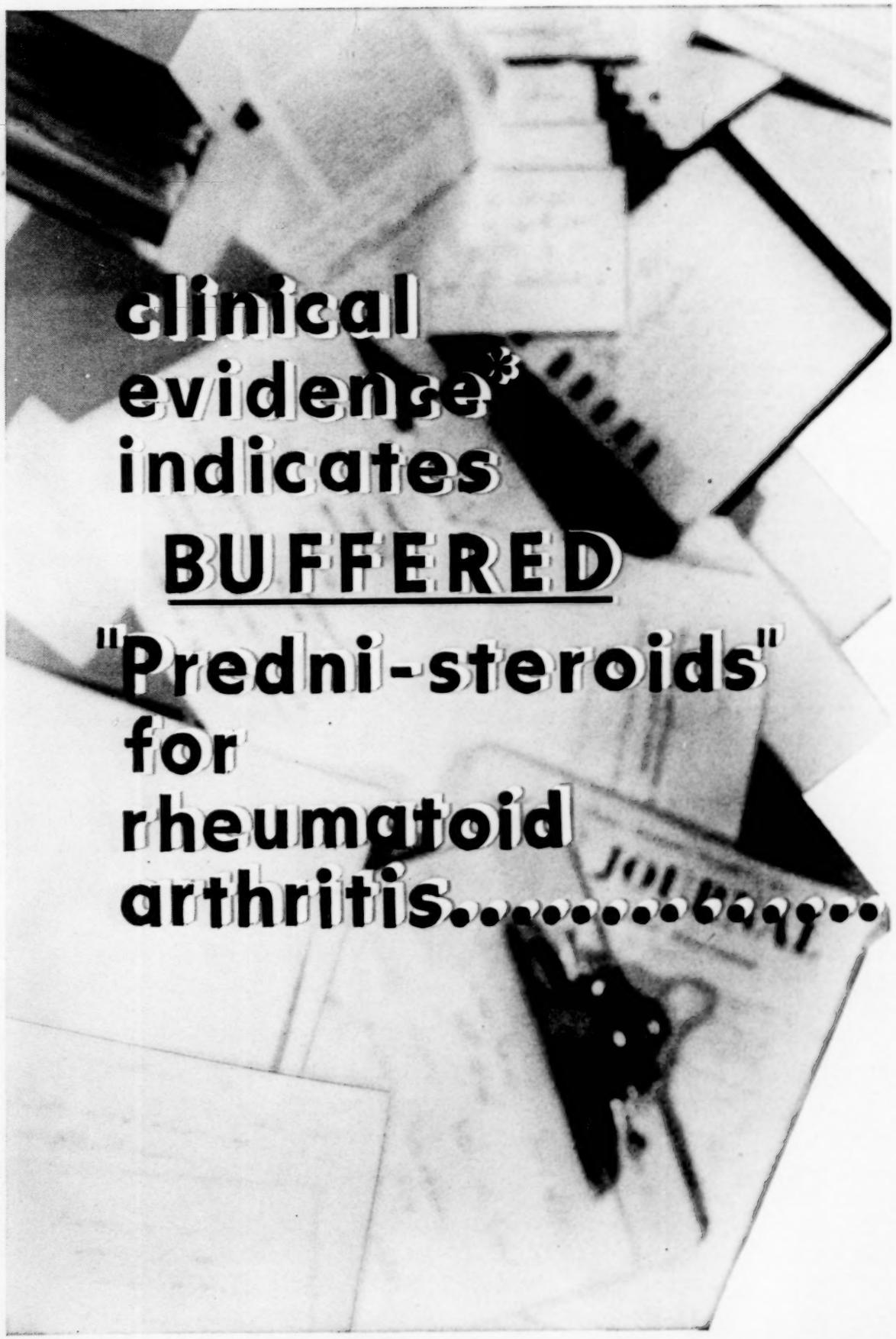
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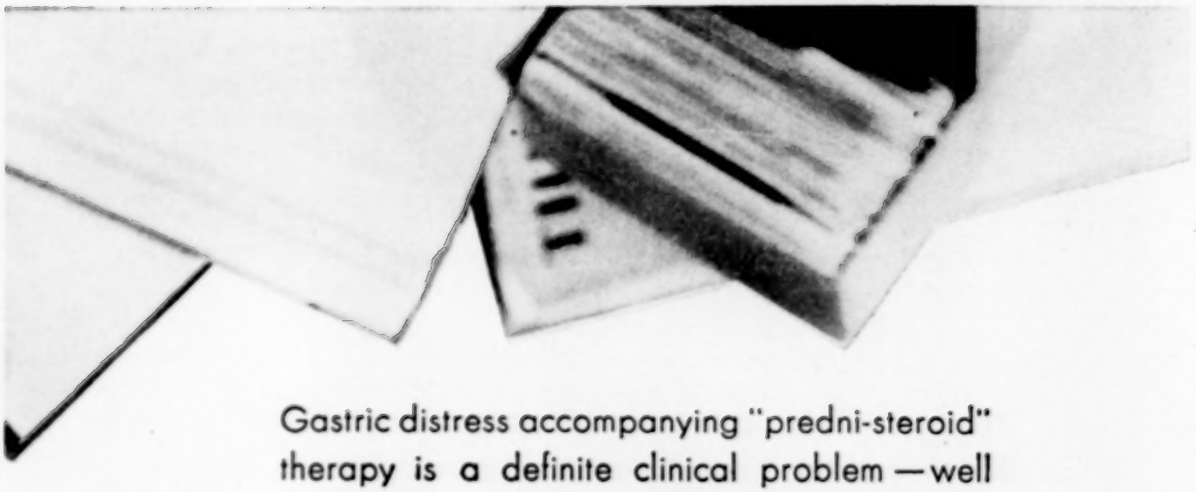
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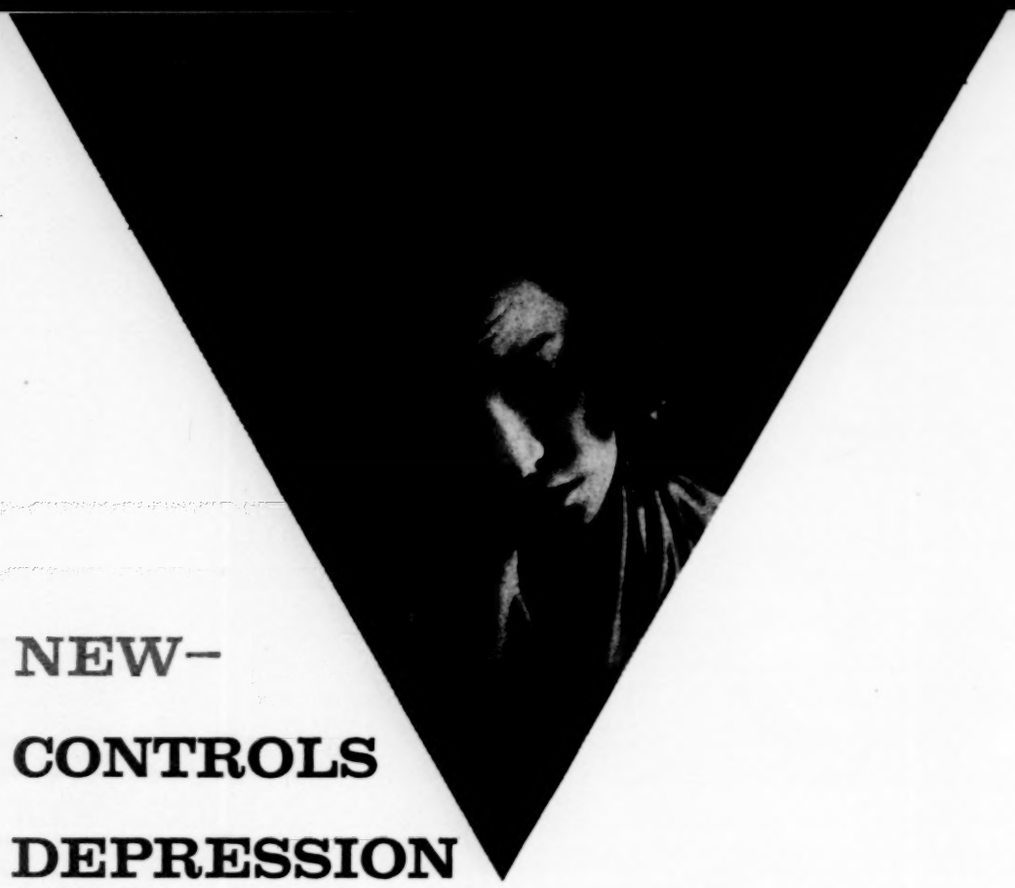
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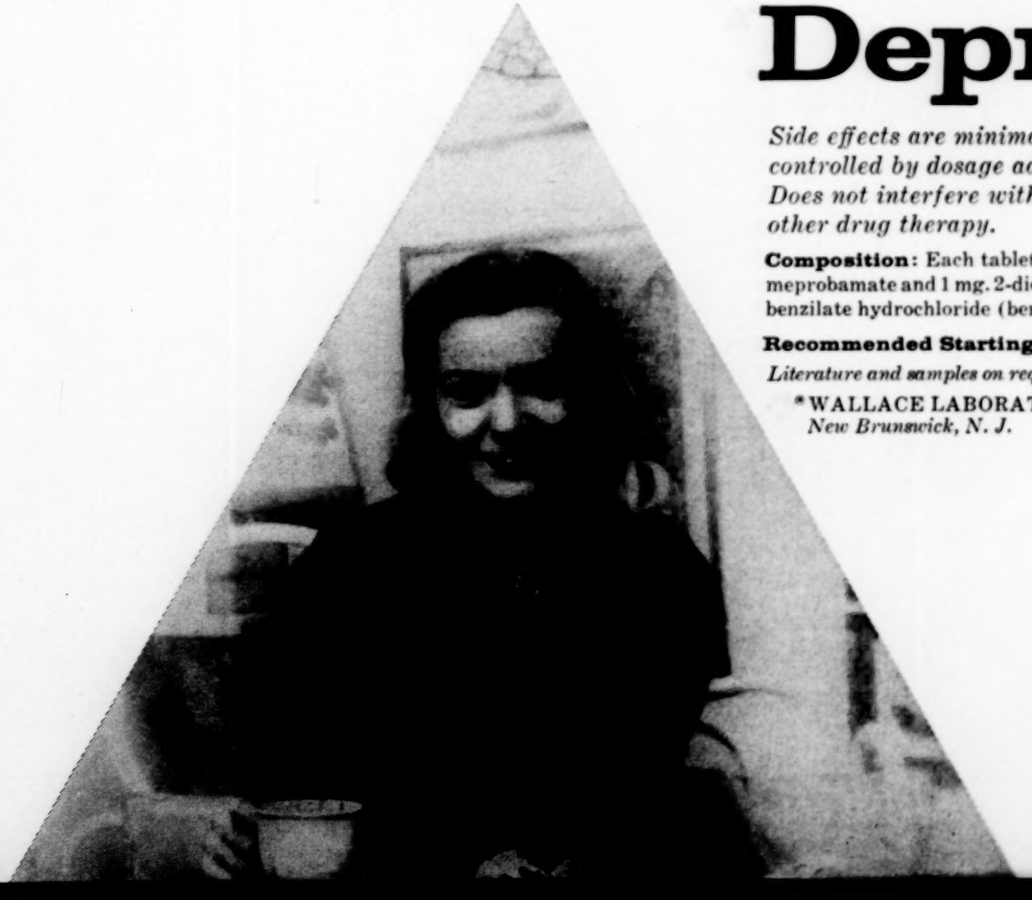
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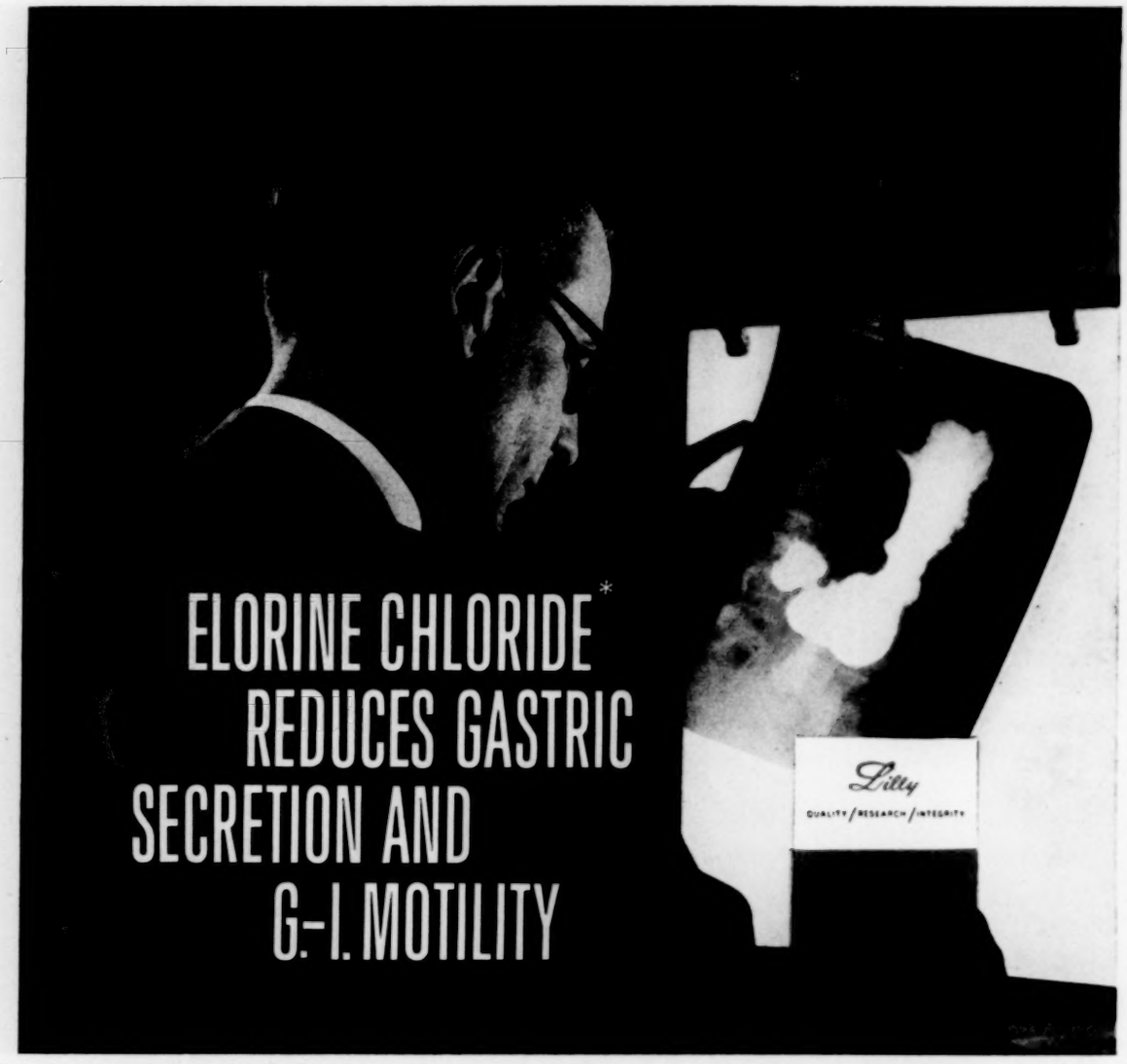
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1. Sun, D. C. H., and Shay, H.: A.M.A. Arch. Int. Med., 97:442, 1956.

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Foreword

Introduction to a Symposium on the Kidney

On the whole the increase in knowledge within any one of the scientific disciplines appears at times to be depressingly slow. For this reason, some purpose may be served by reviewing briefly the interests which preoccupied the contributors to the Seminars on Renal Physiology published in this Journal in 1950. The views expressed at that time can be compared with those contained in the present symposium and in one which appeared recently in the British Medical Bulletin [1]. Considerable progress is apparent in at least four areas: the morphology and cellular fine structure of the nephron, concepts in the clinical appraisal of renal disorders, descriptive electrolyte physiology, and the cellular mechanisms which operate in renal tubular transport.

The microdissection of mammalian nephrons, a technic introduced some years ago by Oliver, has provided a new perspective in the study of the architecture of the kidney. This approach, discussed at some length in the earlier symposium, continues to throw new light on the structural derangements of the nephron in such diverse clinical disorders as acute renal failure, the Fanconi syndrome and potassium depletion. The recent application of electron microscopy to the study of the kidney has revealed fine structures of unsuspected complexity. It seems clearly evident that these structures should be given serious consideration in future attempts to formulate mechanisms of renal function. Dr. Rhodin, who represents one of the most active groups engaged in electron microscopic studies of the kidney, presents some of the recent contributions in this field, with particular emphasis on the structure of the tubule. Of clinical importance is the recent development of renal biopsy procedures which are useful in diagnosis and in the study of the natural history of diseases of the kidney. The contributions of this technic and an appraisal of its future role in medicine are discussed by Dr. Brun and Dr. Raaschou.

The development of clearance technics by Dr. Homer Smith and others has permitted the precise measurement of certain discrete renal functions. For a number of years, the evaluation of renal tubular activity rested on the assumption that a given substance could be filtered by the glomeruli and either reabsorbed or secreted by the tubules. When the amount excreted was less than the amount filtered, it was assumed that the remainder had been reabsorbed; excretion of more than the amount filtered indicated tubular secretion. Hence, the magnitude of tubular transport was a matter of simple calculation. The demonstration in 1949 that potassium can be both reabsorbed and secreted by the renal tubule seemingly introduced overwhelming complications into the interpretation of clearance data. Nevertheless, further investigations into the mechanisms which operate in the renal handling of electrolytes have yielded some measure of clarification rather than increasing confusion. Eight years ago, Berliner's review on the Renal Excretion of Electrolytes considered the problem of sodium excretion primarily in terms of glomerular filtration and reabsorption by tubular mechanisms, the number and inter-relationships of which remained obscure. Although ion-exchange mechanisms had been demonstrated to participate in the acidification of the urinary buffers and in the tubular secretion of potassium, these were regarded more as isolated processes than as parts of an integrated mechanism for sodium reabsorption. In the intervening years, the introduc-

[1] Symposium: Physiology and pathology of the kidney. *Brit. Med. Bull.*, 13: 1–70, 1957.

tion of potent carbonic anhydrase inhibitors has led to certain unifying concepts. Although approximately 80 per cent of the filtered sodium is reabsorbed in association with chloride by mechanisms which are still poorly understood, the remaining 20 per cent appears to be adequately accounted for by the interrelated ion-exchange mechanisms which underlie the reabsorption of bicarbonate, and the excretion of titratable acid, ammonium and potassium ions. In his present review, Pitts summarizes the mechanisms of action of a variety of diuretic agents. In addition, he presents new information on the transtubular potential and data obtained by the "stop-flow" analysis technic of Malvin, Sullivan and Wilde. Dr. Pitts proposes a speculative and provocative scheme to account for the reabsorption and secretion of the principal electrolytes in both the proximal and distal tubules.

The clinical appraisal of renal function necessarily entails the consideration of many possible types of dysfunction, both glomerular and tubular. Until fairly recently most surveys of renal function in disease concerned themselves largely with estimates of the glomerular filtration rate, the renal blood flow and the maximal rates of transport (Tm) for such substances as glucose, diodrast and p-aminohippurate. Attempts were made to interpret certain phenomena of disease in terms of the interrelationships between these measurements. For example, it seemed plausible that the "glomerular-tubular imbalance" in certain states of sodium and water retention might be profitably explored by measuring the glomerular filtration rate and a representative tubular activity, such as diodrast Tm. The limited success of this approach reflects the oversimplifying assumption that one tubular function can be equated with another unrelated function. There is a growing awareness that "tubular function" comprises a host of discrete processes, many of which operate independently and are spatially separated in their localization within the nephron. It is worthy of comment that much of our current knowledge of certain transport mechanisms has been obtained from the study of clinical disorders. This is particularly true of the renal aminoacidurias, the study of which has disclosed the multiplicity of mechanisms which are responsible for the tubular reabsorption of the various amino acids. A number of tubular dysfunctions of clinical interest are reviewed by Mudge, who emphasizes the view that the study of these conditions will continue to offer a profitable avenue of approach to an understanding of fundamental physiologic mechanisms.

Clinical and experimental disorders of potassium balance have been of increasing interest, not only in relation to the renal mechanisms involved but also to the role of potassium in regulating acid-base balance in the various fluid compartments of the body. The syndrome of severe potassium depletion is discussed by Relman and Schwartz with emphasis on certain other secondary effects on renal function, particularly those relating to water balance.

There is another aspect of the interpretation of clearance data which may require some degree of reappraisal. Not infrequently the values obtained by clearance technics are dealt with as though they reflected the activity of a single giant nephron, rather than an aggregate of some two million anatomically and functionally individual units. Bradley and Wheeler consider the evidence arising from a number of lines of investigation that suggests some degree of heterogeneity among the nephron population.

The terms tubular reabsorption and tubular secretion, as commonly used, denote simply the movement of substances across the tubular epithelium in a certain direction without implying the nature of the underlying mechanism. Three types of mechanisms are considered in the present symposium. The first, "active transport," requires that work be done by the cell to move a substance across the tubular epithelium against a chemical potential gradient. Taggart reviews recent attempts to relate specific biochemical events within the cell to the active transport of representative organic compounds. A second type of mechanism is discussed by Milne, Scribner and Crawford who deal with the passive diffusion of un-ionized weak acids and bases across the tubular epithelium. Finally, the diffusion of water along osmotic gradients established by the active transport of solutes is considered by Berliner, who summarizes current concepts of urine concentration and dilution mechanisms. The anatomical relationships which he discusses re-emphasize the importance of attempting to relate structure and function.

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Symposium on Renal Physiology

Electron Microscopy of the Kidney*

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THE structure of the nephron includes a great variety of cell types, from the complicated composition of the filtering glomerular capillary membrane to the relatively simple and pale cells of the collecting ducts. All the different kinds of cells have been studied extensively with the light microscope for a great many years, and various functions have been assigned to different parts of the nephron, mainly as a result of the morphologic studies.

The utilization of new technics in morphology, such as phase contrast microscopy, fluorescent microscopy, polarization microscopy, and especially electron microscopy, has contributed a great deal to our knowledge of the fine structure of the epithelial cells of the nephron. By and large, the structures seen in the electron microscope have all been analyzed with the light microscope, but there are, at present, more detailed and accurate descriptions available which make it possible to draw conclusions more precisely than before as to possible correlations between structure and function.

Hence, some recent findings concerning the fine structures of the nephron will be considered, and the possible role of these structures in the formation of urine will be discussed.

MATERIALS AND METHODS

In most cases kidney tissue from the mouse [*29,38*] and rat [*2,4,12*] has been investigated. Some investigations have also dealt with the dog [*2,7,8*], rabbit [*2,7,8*], and man [*5*]. No great differences seem to have been revealed as far as the fine structure of the nephron is concerned.

The resolution limit of the light microscope is approximately 2,000 Å, whereas the theoretical resolution limit of the electron microscope is about 2 to 4 Å. However, to be able to utilize the resolution of the electron microscope, the specimen investigated must be extremely thin, that is, between 100 and 500 Å in thickness. This was a problem for twenty years, as the first electron microscope was designed around 1932, and useful sectioning technics were not developed until 1952. Before this development one had to fractionate the cell and analyze isolated cell organelles such as nuclei, mitochondria, and microsomes. However, this technic did not contribute to the knowledge of the fine structure of the cytoplasm, the intracellular relationship between various cell organelles, or to the fine structure of their interior. Information of this kind awaited the development of proper sectioning technics [*23,33*], a new embedding material [*13*], and fixation methods [*17,34*] contributing to good preservation of the tissue.

In short, the common way of preparing tissues for electron microscopy today includes fixation in osmium tetroxide which simultaneously serves as an electron stain. Osmium tetroxide is not a selective stain, but it may be considered that protein structures are stained more heavily than lipids, whereas carbohydrates do not take up osmium at all. Following fixation, the tissue is embedded in liquid plastic, which thereafter is hardened by adding a polymerizer. Sections with a thickness of about 100 Å are then cut with special microtomes, and can immediately be investigated in the electron microscope without removing the embedding medium.

Artifacts may be caused by postmortem processes, or by improper temperature, pH, and salt concentration of the fixative. Additional distortion of the tissue may also occur during polymerization of the plastic. The types of damage caused by any of the preparation steps mentioned is fairly well known [*21,34,42*], but they can be avoided. Consequently, such artifacts are not the kind which would seriously affect the interpretations presented here.

GLOMERULUS

The capillaries of the glomerulus are abundant and branch off from the afferent vessel in main stems (Fig. 1, C1 and C2) which again come together and form the efferent arteriole. By microdissection of glomeruli injected with latex particles, Hall [*7,8*] has been able to prove the concept held by Bowman that there are

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FIG. 1. Glomerulus of the mouse kidney. Electron micrograph. Fixation buffered osmium tetroxide. Magnification × 16,000.

small vessels which take off at right angles from the larger vessels, thus interconnecting these with each other. Hall considers this of importance in the formation of urine, and believes that the blood cells follow the main branches, whereas most of the plasma passes into the

smaller branches. From a functional point of view this could mean that the plasma volume is increased within the numerous small capillaries of the glomerulus, and thus the contact area between plasma and filtering membrane also becomes increased.

The structure of the filtering membrane is rather elaborate and complicated. It consists of the inner endothelial layer, the outer epithelial layer, and a basement membrane in between.

The cells of the endothelial layer are of a squamous type with a light cytoplasm and an elongated nucleus (Fig. 1, Nep) which bulges into the capillary lumen. Only a few mitochondria are scattered in the light cytoplasm. This is to be expected in a cell in which little enzymatic activity is required for the filtering process. The cytoplasm also contains a small Golgi zone, and occasional α-cytomembranes [*35*], features which are more necessary in cells with high secretory activity, such as pancreatic cells [*36*] and mucus-producing cells [*29*].

The cytoplasm is attenuated, forming a thin layer which lines the inside of the capillary wall. The thin layer has a thickness of about 500 Å in its extreme, and displays a great number of perforations (Fig. 1, arrows) with a diameter ranging between 500 Å and 1,000 Å. These perforations represent true holes in the endothelial cytoplasm [*7,8,20,21,25*] though it has been suggested that they are closed by a thin membrane [*41*].

The central part of the filtering membrane is formed by the basement membrane, (Fig. 1, E) which is a continuous, structureless layer about 800 Å thick. Like other basement membranes analyzed with the electron microscope, it seems to be composed of mucoproteins, possibly with fibrillar subunits [*25*] although most investigators believe it to be definitely homogeneous [*7,8,20,21*].

The outer epithelial layer is the most intriguing of all layers of the filtering membrane, and consists of big cells with a denser cytoplasm than the endothelial cells. The bulge, corresponding to the site of the nucleus (Fig. 1, Nep), is heavier, and incorporates also a large part of the cytoplasm. The cell resembles a jelly fish, as the cytoplasm is extended into long tentacles which grasp the capillary wall. The tentacles have been called trabeculae, and these in turn give rise to smaller tentacles, the so-called pedicles (Fig. 1, P) which branch off perpendicularly to the direction of each trabecula and interdigitate with each other on the surface of the capillary. There is only a short distance between different pedicles, and according to a theory recently put forward by Hall [*9*], this distance, or slit-pore, is the ultimate part of the filtering membrane that determines the size of the molecules which will be allowed to filter through the membrane. The smallest distance measured by Hall [*9*] averages 70 Å, which is approximately the size of a hemoglobin molecule. If the slit-pore is widened only slightly, it will allow larger molecules to penetrate the wall and reach the space of Bowman's capsule. (Fig. 1, B.) This widening may take place whenever the normal metabolism of the epithelial cell cannot be maintained by the enzyme system present in the mitochondria of the cell.

PROXIMAL CONVOLUTION

The inner surface of Bowman's capsule is lined by squamous cells which are continuous with the tubule cells lining the neck and the proximal part of the nephron. The capsular cells are light and simply constructed, whereas the tubule cells (Fig. 1, Tb) are dense and display a complicated structure. The capsular cells rest on a basement membrane (Fig. 2, E), which is continuous with the membrane surrounding the nephron in its whole length, and extends down to the collecting tubules and the pelvic papillae.

In tissues well fixed in osmium tetroxide [*3,24,38*], as well as in frozen dried material [*32*], the lumen (Fig. 2, L) of the nephron is usually closed, presumably due to a certain collapse shortly after the animal is killed. The central part of the tubule is thus occupied by a multitude of densely packed microvilli, or brush border extensions. Each microvillus has a length (Fig. 2, Bl) of about 1 micron and a width (Fig. 2, Bt) of about 700 Å. There are roughly 215 microvilli per square micron in the first part of the proximal tubule. However, the number of microvilli decreases towards the thin segment of Henle's loop. A distinct cell membrane with a thickness of 40 Å separates the lumen of the tubules from the interior of the microvillus which is connected freely with the rest of the cell cytoplasm. The interior of the microvillus is structureless, and less dense than the rest of the cell cytoplasm, which may imply that this part of the cell contains a special enzyme system or is the site of special enzymatic activity involved in the phosphorylation of



FIG. 2. Proximal convoluted tubule cell of the mouse kidney. Electron micrograph. Fixation buffered osmium tetroxide. Magnification × 19,000.

glucose, as this substance is reabsorbed entirely by the cells of the proximal convoluted tubule.

At the bases of the microvilli, small holes open into coiled tubular invaginations (Fig. 2, Ti) of the surface membrane of the cell. The width of each invagination is about the same as that of a microvillus, whereas the length is shorter. The invaginations are sometimes widened to small

bulbs, but they do not seem to be connected with the large vacuoles which occur in the apical cytoplasm. The function of these invaginations is still unknown, but they may be involved either in reabsorption or secretion of certain substances. The nearby large vacuoles (Fig. 2, V) correspond to autofluorescent granules which Sjöstrand [*32*] observed in sectioned and unstained material, prepared by the freeze-drying technic. They probably contain fluid material which becomes washed out during the fixation and embedding procedure for electron microscopy. In close relation to the fluorescent granule vacuoles are encountered large electron dense granules (Fig. 2, Dg) which often display a system of concentrically arranged thin membranes.

It is most likely that the fluorescent granule vacuoles and the large electron dense granules represent features of the proximal tubule cells which should be considered as secretion products, as it is often noticed that the large electron dense granules occur in the tubule lumen. A possible continuation between the large electron dense granules and the fluorescent granule vacuoles may also exist, accomplished by a condensation of the vacuoles into dense granules. This is not merely a hypothesis, as forms transitional between these two cell organelles often occur. However, it must be considered that the direction of the event could as well be the reverse.

As mentioned, the Golgi zone is also most likely involved in the secretory activity of cells. However, this is more obvious in typical gland cells, such as pancreatic cells [*19,37*] and the goblet cells of the intestine or the respiratory tract [*29*]. In the cells of the proximal tubule, there is also a Golgi zone (Fig. 2, Ga), lying like a halo around the apical part of the nucleus. It is composed of a system of paired membranes parallel to each other, with small vesicles scattered either between the paired membranes or peripherally.

The activity of the Golgi zone in tubule cells seems to be concerned with intracellular events. Eventually some of the small vesicles become condensed, and appear as electron dense granules, bordered by a single membrane. Similar granules, but of varying size, may be encountered in close relation to the Golgi zone. They never achieve the size of the large electron dense granules previously mentioned, and they also differ from them in that the large granules are bordered by a triple-layered membrane with a total thickness of 100 Å, whereas the small granules are bordered by a single membrane 45 Å thick. The fate of the small granules derived from the Golgi zone is still unknown, but it may be suggested that the bigger ones are identical in size and shape with particular cell organelles called microbodies. The microbodies (Fig. 2, Mb) are spherical, with a diameter of about 0.2 micron and with the same electron density as the mitochondria. They were first encountered and analyzed in the tubule cells of the mouse [*24*] and rat [*6*] kidney, and have recently been found also in the liver cells [*30*]. The microbodies do not represent the "microsomes" of a cell, as microsomes is a name applied to particles of a certain size which can be spun down at a certain speed in a centrifuge after the cell has been fractionated. Thus, microsomes may be derived from any cell organelles which are broken into pieces during fractionation. The microbodies are surrounded by a single membrane 45 Å thick, and lack inner membranes. They seem to constitute precursors of mitochondria in the cells of liver and kidney tubules. Thus, it seems likely that one task of the Golgi zone in the cells of the proximal tubule is to provide the necessary supply of mitochondrial precursors. A strong support of the hypothesis that microbodies develop into mitochondria is found in experimental hyperparathyroidism [*4*]. After injection of parathormone into rats, the cells of the proximal tubule of the kidney display a reduced number of mitochondria, whereas the number of microbodies is markedly increased. This may indicate that the normal development of mitochondria is blocked. As a result of this, the normal supply of enzymes delivered by the mitochondria is decreased, and the normal cell metabolism altered, which results in the formation of intracellular hyalin bodies. These bodies become enlarged, occupy the whole cell, burst into the lumen of the tubules, and form hyalin casts which later serve as centers for the precipitation of calcium salts (apatite crystals) during the formation of renal calculi.

The mitochondria (Fig. 2, M) of the cells of the proximal tubule occupy the largest part of these cells, and form elongated, sausage-like, coiled bodies. The fine structure of a mitochondrion consists of a surrounding triple-layered membrane, and a system of triple-layered membranes, arranged parallel to each other in the interior of the mitochondrial body,

mainly perpendicularly to the long axis of the mitochondrion [*24,38*].

About 90 per cent of all enzymes in the tubule cells are located in the mitochondria. The inner membranes, or plates, presumably represent the site of the enzyme molecules, or the surfaces upon which part of the enzymatic activity can occur. This fine structure is probably necessary to maintain the normal function of a mitochondrion. This has been demonstrated in connection with the injection of egg white into the abdomen of mice [*24*]. The egg white, absorbed by the peritoneum, enters the blood, and reaches the glomerulary capillaries, where the protein molecules are small enough to escape through the filtering membrane [*15*]. Now, in the lumen of the nephron, the filtered egg white becomes reabsorbed by the cells of the proximal tubule. During this absorption, a certain reaction seems to occur between the egg white and the mitochondria; the latter swell and lose their typical normal fine structure, which is replaced by an electron dense, structureless substance. Simultaneously, a decrease in sodium and water reabsorption can be recorded in the experimental animals. As is known, most of the water and sodium is reabsorbed by the cells of the proximal tubule, activities that by all means require enzymes and energy. It is most likely that the tubule cells with their altered mitochondria have lost the ability of generating energy for the osmotic work of the cells.

As just mentioned, most of the water reabsorbed by the tubules must pass through the cells of the proximal tubule of the nephron. It probably enters the cell through the cell membrane which covers the microvilli (Fig. 2, Bl), although the tubular invaginations (Fig. 2, Ti) may also be utilized in this process. The pathway of the water through the tubule cell is not to be found as a structural canal, or anything similar. However, the basal part of the cell is designed in a special way which may help to facilitate the passage of water from the interior of the tubule cell to the capillary wall which is connected to the basement membrane of the nephron. The basal part of the tubule cell is provided with a multitude of cytoplasmic lamellas (Fig. 2, Cl), an arrangement resembling the cone-shaped cogwheel in a differential gear of a car, except that the lamellas of this particular cogwheel are higher near the top of the cone than they are at the base of the cone (Fig. 6). Of course, the lamellas of the tubule cell are more flexible, and subject to more variations than those of a cogwheel. The way the basal parts of different tubule cells fit together, is much the same, however, as the manner in which the cogwheels of a differential gear interlock. This organization* of the bases of the tubule cells means, of course, that the basal surfaces of the cells are tremendously increased. As a result of this lamellation, the basal cell membrane will be encountered also at varying distances within the cell body, as seen in a section of a cell. It may be suggested that these deep infoldings of the basal cell membrane serve as surfaces upon which enzymatic activity can take place, and that the water may use this increased basal surface for easy and rapid access into the extracellular, or intercellular space, and from there reach the capillary wall only by passing the basement membrane of the tubule. Furthermore, the mitochondria are almost exclusively located within the basal cytoplasmic lamellas, in close contact with the cell membrane, which strongly supports the idea that the multifolded cell membrane is designed in this way in order to increase the surface upon which enzymatic activity can occur. The lamellation of the cells of the proximal tubule is to some extent similar to the trabeculation of the epithelial cells of the glomerular capillaries [*2,7,8*], but the lamellae of the tubule cells do not seem to be divided into smaller subunits like pedicles (Fig. 1, P) to any greater extent. The regularity with which the pedicles project at right angles and interdigitate with each other has not been seen in the cells of the proximal tubule. Nor has it been possible to confirm the findings by Ruska et al. [*31*] and their three-dimensional interpretation of the membranous structures in the tubule cells of the kidney in the rat.

The sides of the upper portion of the cells are smooth, without the typical basally located lamellas. In transverse sections through this region, each cell displays a multiangular shape, while in sections through the basal part it appears as an irregular, multipointed star. Neighboring cells stick to each other probably by the complicated interlocking of the basal

* The interpretation of the arrangement of the cytoplasmic lamellas in the cells of the proximal tubules was presented by me before the meeting of Deutsche Gesellschaft für Pathologie in Bad Nauheim, Germany, April 10, 1957. The report has appeared in print elsewhere [*27*].

cytoplasmic lamellas. Close to the surface of the cell, however, each cell is strengthened and attached to another by the so-called terminal bars which represent a local thickening of the cell membrane, oriented as a coopering band around the top part of the cell, and almost completely filling the space between the cell borders, a space which averages 70 Å and probably is composed of at least two layers of lipoid molecules.

The nucleus (Fig. 2, N) is located in the center of the basal part of the cell, which means in the top of the cone-shaped cogwheel. The nucleus does not go into the cytoplasmic lamellae. A triple-layered membrane with a thickness of about 250 Å surrounds the nucleus. One or several nucleoli are present, composed of fine granules, as is the rest of the nuclear content.

The structure of the cytoplasmic ground substance is finely granulated (Fig. 2, *) with particles averaging 160 Å. Particles of similar size have elsewhere [*18,19*] been proved to contain ribonucleic acid (RNA).

THIN SEGMENT OF HENLE'S LOOP

The cells of the descending limb of Henle's loop are different from those of the proximal part of the convolution. As previously mentioned, the number of microvilli decreases. Furthermore, the mitochondria are less abundant and shorter, more like spheres. The cytoplasm itself is less dense, and the basal cytoplasmic lamellas are rare, sometimes absent [*26*]. The height of the cells becomes more shallow, and in the thin segment the cells are of a definite squamous type [*20*].

As in most squamous epithelia, the nuclei cause the top part of the cell to bulge, in this particular case into the tubule lumen (Fig. 3, L). The surface of the cell is fitted with tiny microvilli (Fig. 3, Mv), spaced apart at a distance of about 0.1 micron. The surface of the villi (Fig. 3, inset, Mv), and that of the areas between them, is covered with a single membrane, 70 Å thick. Invaginations of the membrane seem not to occur in this part of the nephron. The cytoplasm is pale, and holds only a few scattered, small mitochondria (Fig. 3, inset, M) together with a very restricted Golgi zone, features encountered mostly in the neighborhood of the nucleus.

The cytoplasm of the squamous cells is extremely attenuated. The height varies, of course, depending on the distance from the nucleus (Fig. 3, N), but the thinnest parts average 0.5 micron. The attenuated cytoplasm is composed of lamellas (Fig. 3, arrows), arranged in such a way that the cells of the thin segment resemble starfishes (Fig. 6) with a multitude of arms resting on the basement membrane (Fig. 3, E). The arms do not have a straight course, but a highly curved and wavy one, and the arms or lamellas of one cell interdigitate with the lamellas of neighboring cells. This is similar to the arrangement of the cells of the proximal tubule, only with the exception that the cells of the thin segment are flattened down, so that the upper part of the thicker portions of the cells are not in contact with each other. The cytoplasmic lamellas of the cells of the thin segment are rather narrow, and quite frequently contain mitochondria. The lamellas are surrounded by a cell membrane, which is provided with terminal bars (Fig. 3, inset: arrows) near the luminal (Fig. 3, L) surface.

The organization of the attenuated processes of the cell body, interdigitating and resting on a basement membrane, is almost identical with the picture of the pedicles of the glomerular epithelial cells. This would imply that fluid in the tubule lumen has a more or less free admittance between the cytoplasmic lamellas to the capillary wall, which is connected to the outer surface of the thin segment. However, one has to keep in mind that the pedicles of the glomerular epithelial cells are not connected by terminal bars which is the case in the cells of the thin segment. Until further information can be obtained as to the function of the terminal bars, one has to consider them as a rigid, supporting part of the cell wall with a connective as well as a blocking function preventing the cells from separating, and hindering fluids or other substances from using the intercellular space as a pathway.

The organization of the cells rather suggests that they function like ordinary capillary endothelial cells. It is believed that "the thin segment is concerned with passive osmotic dialysis of the tubular urine to bring it to an isoosmotic state relative to the blood before it enters the distal tubule" [*39*]. Thus, the osmosis does not require energy to a greater extent, which tallies well with the relatively small number of mitochondria present in the cells of the thin segment. On the other hand, the multitude of cytoplasmic lamellas may imply that the osmosis is facilitated by the enlarged intercellular surfaces.

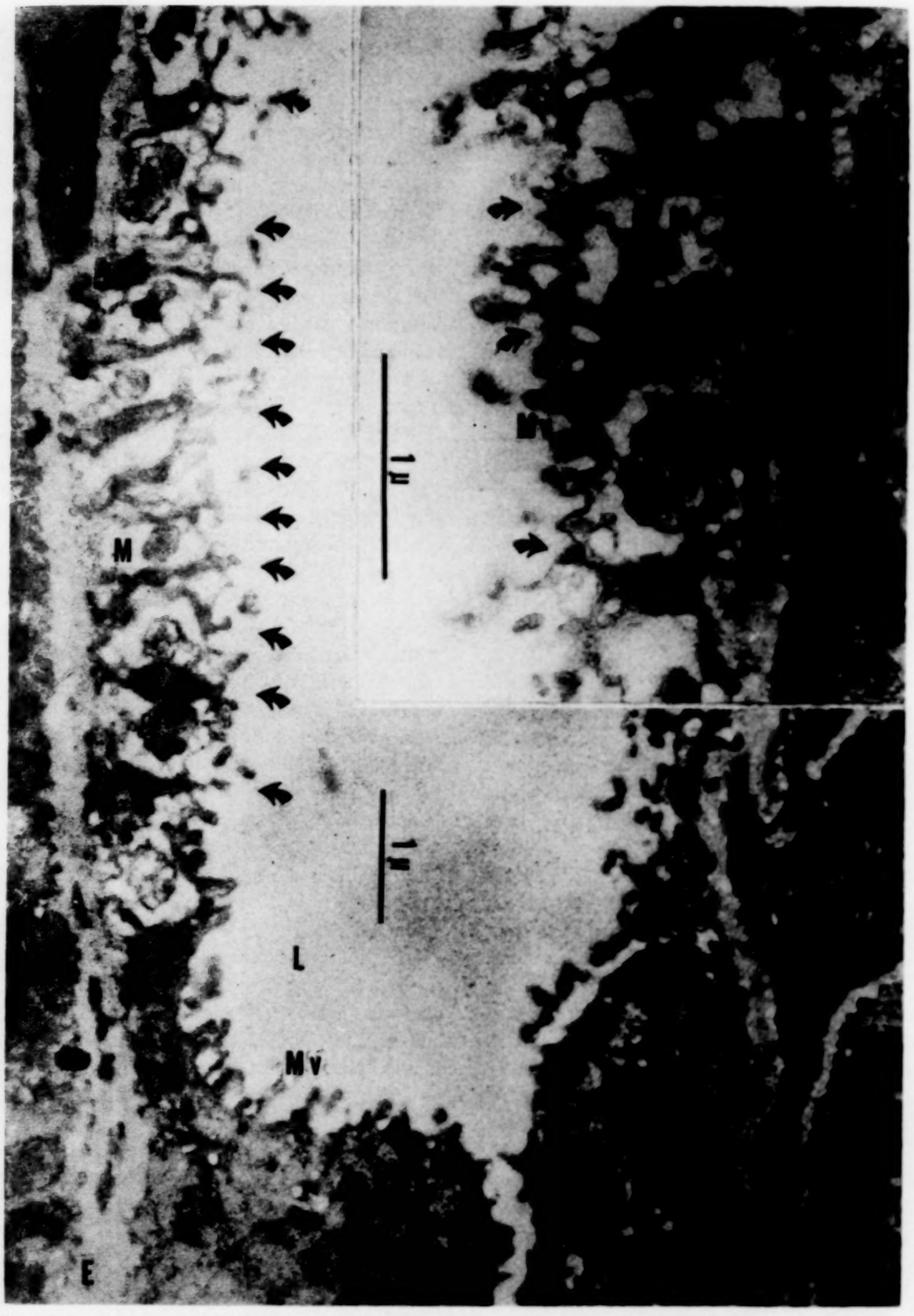


FIG. 3. Thin segment of Henle's loop of the mouse kidney. Electron micrograph. Fixation unbuffered osmium tetroxide in 12 per cent dextran. Magnification × 18,500; inset × 31,500.

DISTAL CONVOLUTION

In the first part of the ascending limb of Henle's loop the cells are cuboidal and characterized by a rather clear cytoplasm, resembling that of the cells of the thin segment. However, as the neighborhood of the glomerulus is approached, the cells become more columnar, the cytoplasm darkens, and the mitochondria in-

crease in number and length. The long mitochondria are particularly typical of this part of the nephron; they contribute to a marked basal striation, observable even in the light microscope. The diameter of the tubule is larger than in the proximal convolution, and a wide, free lumen (Fig. 4, L) is always present [*22*].

There is a complete lack of a true brush border, but a few small microvilli (Fig. 4, Mv), with a length of about 0.4 micron, are scattered over the surface of the cells of the distal convoluted tubule. The microvilli, as well as the free cell surface, are covered by a cell membrane 70 Å thick.

The luminal one third of the cell is characterized by an abundance of small vesicles (Fig. 4, arrows). Those located close to the surface of the cell are now and then in open connection with the tubule lumen (Fig. 4, L), and may represent structures of the same nature as the tubular invaginations of the cells of the proximal tubule. If this is the case, the rest of the vesicles can be interpreted as sectioned tubular invaginations with a highly coiled, spring-like course. However, it is more likely that the vesicles represent isolated profiles of structures which take part in a secretional activity of the cells of the distal tubule, either in connection with the acidification of the urine, or with the formation of ammonia, which is synthesized and secreted by the cells of the distal tubule.

A certain number of fluorescent granule vacuoles (Fig. 4, V), as well as large electron dense granules (Fig. 4, Dg), are present, but to a lesser extent than in the cells of the proximal tubule.

There is a Golgi zone (Fig. 4, Ga) with a location and fine structure similar to that which has been described in the proximal convolution. However, the vesicles seem to be larger in the distal convolution, almost achieving the size of the fluorescent granule vacuoles. A transition of Golgi zone vesicles into condensed bodies, resembling microbodies, has not been recorded.

A certain number of microbodies occur, but with less abundance than in the proximal convolution. It must be stressed in this connection, that the possible transformation of microbodies to mitochondria is still a problem to be definitely solved. The mitochondria have a triple-layered outer membrane and the microbodies a single-layered. The transformation could be accomplished by an addition of membranous structures to the outside of the microbody. Indications of a similar mechanism have been observed in areas in which the ground substance of the cytoplasm displays a certain membranous structure, in addition to the previously mentioned RNA-granules. Eventually, these membranous sheets (Fig. 2, **), with a thickness of 45 Å and a length of 0.1 to 0.2 micron, are observed partly to encircle the microbody, giving the impression of a local triple-layering of the microbody wall.

The mitochondria (Fig. 4, M) of the cells of the distal convoluted tubule have a length of about 3 microns or more, and their width averages 0.3 micron in one direction, whereas their thickness amounts to, or even exceeds, 1 micron. In consequence of this, the mitochondria have a closer resemblance to flat ice cream bars than to sausages. This is true for the mitochondria which occupy the basal two-thirds of the cell. In the luminal one-third, small spherical or slightly oval mitochondria are seen scattered between the abundant small vesicles already mentioned. The basal mitochondria are tightly packed, sometimes so tightly that the interposed narrow strands of cytoplasm are hard to distinguish. The fine structure is almost identical with that described for the mitochondria of the proximal convolution. One exception may be stressed, however. The inner, triple-layered membranes, or plates, are sometimes seen oriented at various angles to the long axis of the mitochondrion, which seems to indicate that their three-dimensional orientation is a helix, rather than simple straight plates across the interior of the mitochondrial body.

The basal part of the cell of the distal convoluted tubule displays cytoplasmic lamellae (Fig. 4, Cl) similar to those found in the proximal convolution, although the lamellae seem to be narrower, deeper, and of greater abundance in the distal convolution. The interdigitation of lamellas from different cells is identical in the proximal and distal convolutions (Fig. 6).

It is not surprising that the cells of the distal tubule contain a great number of large mitochondria, located within the multitude of narrow cytoplasmic lamellas, when the function of the distal tubule is considered. The final reabsorbtion of water and sodium occurs here, and as the water must be reabsorbed against osmotic pressure, the process probably requires a great deal more energy than in the proximal convolution, where the osmotic pressure of the contained urine remains identical with that of the plasma.



FIG. 4. Distal convoluted tubule cell of the mouse kidney. Electron micrograph. Fixation buffered osmium tetroxide. Magnification × 22,000.

The close relationship between the mitochondria and the cell membrane of the cytoplasmic lamellas seems to facilitate the passage of water from the cell to the capillary in accordance with the discussion herein. The abundance of mitochondria is also associated with the secretory function of the distal tubule, as the synthesis of ammonia and the acidification of the urine probably demands a high enzymatic activity.

The cells of the distal tubule are united by the interlocking of cytoplasmic lamellae. In addition, terminal bars (Fig. 4, T) are present with a structure and location identical with that already described for this cell component in the proximal convolution.

The nucleus (Fig. 4, N) is placed in the luminal part of the cell, and bulges the cell surface into the lumen (Fig. 4, L). Nothing can be added to the description of the fine structure of the nucleus, as compared to what has already been stated concerning the nucleus of the proximal tubule.

The cytoplasmic ground substance is built up of the mentioned RNA-granules, which are abundant in the luminal part of the cell, but almost absent in the basal part, within the cytoplasmic lamellas.

COLLECTING TUBULE

The collecting tubule is not considered to belong to the nephron proper, but now and then certain functions have been supposed, and recent studies seem also to indicate that this part of the kidney is not only a duct for transportation of urine to the renal pelvis, but also an active part of an extended distal tubule.

The collecting tubule is wide, and as the lining cells are rather shallow, this opens a wide and free lumen (Fig. 5, Lu). The cells are mainly of two types, the light cells and the dark, or intercalated, cells. Close to the point where the distal tubule connects with the so-called arched collecting tubule, the light cells are rare, and the dark cells abundant. However, distally in the collecting tubule, within the papilla, the dark cells become more and more rare and the light cells predominate.

The light cells (Fig. 5, L) have a loose cytoplasm due to a scarcity of cell organelles such as mitochondria and cytoplasmic RNA-granules. The nucleus (Fig. 5, N) has a prominent location in the cell, leaving but little cytoplasm free. Between the cells are deep indentations of the surface, which sometimes almost reach the basement membrane. A membrane with a thickness of 70 Å covers the surface of the cell as well as the scattered, few and short microvilli (Fig. 5, Mv), which emerge at a distance of about 0.5 micron from each other. Some small vesicles occur in the apical part of the cell, although their number does not exceed five or so in a section of cell. Occasional fluorescent granule vacuoles and large electron dense granules may be encountered. The mitochondria (Fig. 5, M) are few and located mostly in the basal part of the cell. Their width averages 0.3 micron. The fine structure is identical with that of mitochondria in the proximal tubule. Microbodies have not been encountered in the light cells. The Golgi zone (Fig. 5, Ga) is small but of regular structural appearance. It generally surrounds the upper part of the nucleus, but it may also be more apical in position. The basal part of the light cell shows a few shallow inflections of the cell membrane, which may be interpreted as cytoplasmic lamellae or basal ridges. However, they seldom interdigitate with similar structures from neighboring light cells, probably because they are too short and shallow to penetrate underneath the cells and become interlocked.

The dark, or intercalated, cells (Fig. 5, D), have a very dense cytoplasm, mainly due to an abundance of cytoplasmic RNA-granules, each with a thickness of about 160 Å. The cells show a certain variation in height and form, sometimes being taller than the light cells, sometimes more shallow, with a tendency to extend underneath the light cells, between their cell body and the basement membrane; this may explain why they are said to be intercalated. The surface has an abundance of short microvilli (Fig. 5, Mv), all surrounded by a cell membrane. The luminal one-third of the cell is characterized by a great number of small vesicles (Fig. 5, arrows), some of them in free connection with the lumen (Fig. 5, Lu). Fluorescent granule vacuoles and large electron dense granules are rare. The number of mitochondria (Fig. 5, M) is great in the dark cells of the collecting tubule. They are of spherical shape, the diameter averages 0.7 micron, and the fine structure resembles that of the mitochondria in the cells of the proximal tubule. They are encountered all through the cytoplasm, and sometimes cause heavy bulges of the cytoplasm above the nucleus, an observation

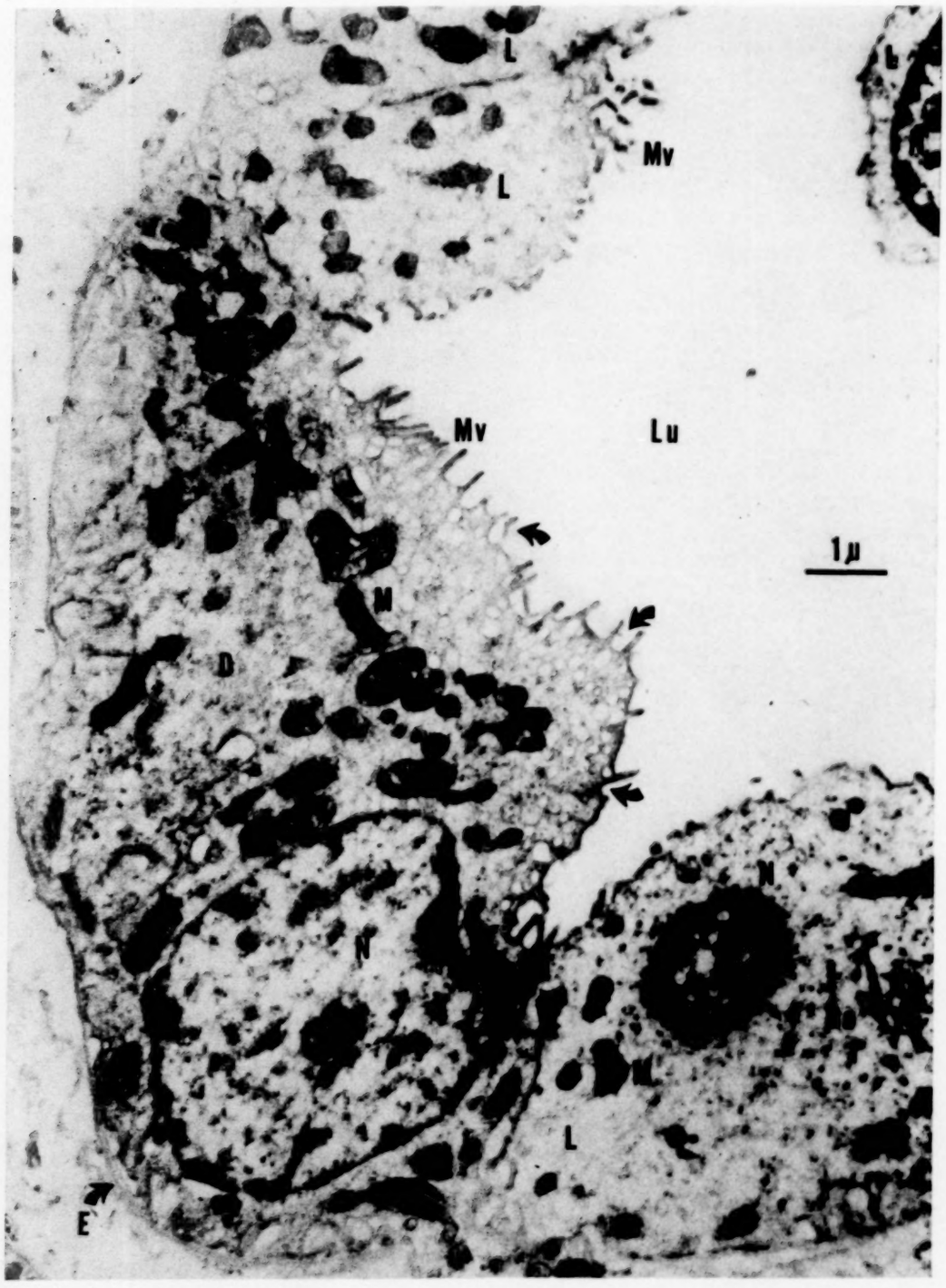


FIG. 5. Collecting tubule of the mouse kidney. Electron micrograph. Fixation buffered osmium tetroxide. Magnification × 11,000.

which can be made even in the light microscope. Quite frequently, a number of microbodies can be seen with a great variation in size. A Golgi zone of common appearance and location is present. Cytoplasmic lamellas are often seen, but they are shallow and narrow. The dark cells do interdigitate with the light cells, but only in the periphery, where the cytoplasmic lamellas are long enough to interlock with each other. This is, of course, especially the case when part of the dark cell is intercalated between the base of a light cell and the basement membrane.

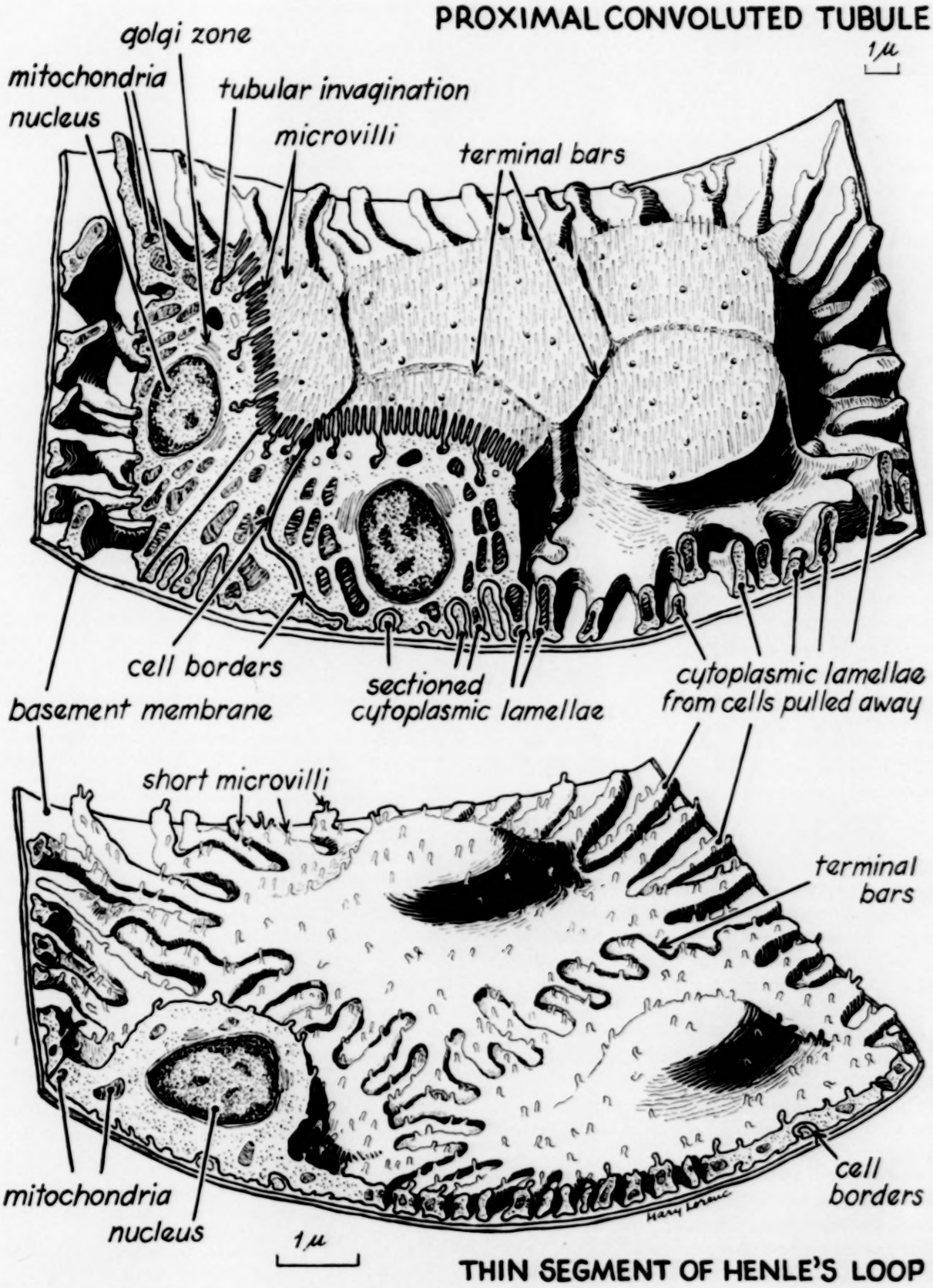


FIG. 6. Three-dimensional reconstruction of the basal interlocking mechanism of cells as analysed in the proximal convoluted tubule and the thin segment of Henle's loop in the mouse kidney.

One function of the collecting tubule without doubt is to serve as a conduit for the urine from the distal convolution to the papillary ducts and openings. Considering the two types of cells present in the wall of the collecting tubule, it may be that the light cells at least serve as a pure lining of this conduit. They may also represent a part of the wall of the tubule through which some water can be reabsorbed during a final concentration activity, a second function of the collecting tubule, suggested by Smith [*40*] and Oliver et al. [*16*]. However, the similarity between the dark, or intercalated, cells of the collecting tubule and the cells of the distal convolution strongly suggests that the former actually represent a certain variety of cells of the distal convoluted tubule, which are distributed along the collecting tubule towards the papilla at a decreasing frequency. If there is any correlation between structure and function, then one would expect that the dark, or intercalated, cells in the wall of the collecting tubule, like the cells of the distal tubule, participate in the secretion of ammonia and in the acidification of urine. These then may be additional functions of the collecting tubule.

The dark cells of the collecting tubule were well described by Andrejewitz [*1*], who also noticed a difference in the shape of these cells, and concluded that this was due to different functional states in a secretory process. In an electron microscopic investigation, Clark [*3*] concluded that the light and the dark cells actually are identical cells, but with a variation in density because of the difference in functional states. The hypothesis put forward by Clark [*3*] seems to be supported by the recent light microscopic investigations by Oliver et al. [*16*]. They recorded an increased number of intercalated cells and a decreased number of light cells in the cortical collecting tubules as a result of potassium depletion in rats. The structural change of the collecting tubules was associated with an inability to concentrate the urine. Whether this inability is due to the increased number of intercalated cells or to the decreased number of light cells is impossible to state at present. At least, it seems to be true, as stated by Oliver et al. [*16*], that "the activity of the nephron may extend beyond the strict limitations of its topographic confines."

CONCLUSION

The purpose of this article has been to review some recent analyses dealing with the fine structure of the nephron. It was impossible to refer to the results of all earlier investigations which employed the light microscope as well as the electron microscope, but the interested reader is referred to a more detailed review of the anatomy of the kidney tubules to appear elsewhere [*28*]. As a morphologist, I am quite aware of my limitations insofar as the evaluation of investigations and theories of renal physiology is concerned.

However, the present suggestions as to the relations between the structures described here and the functions suggested by physiologic experimentation may indicate the type of information which careful investigations with the electron microscope may contribute in the future.

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Kidney Biopsies*

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EIGHT years have passed since the first percutaneous kidney biopsy was carried out. The results of about 1,800 kidney biopsies have now been recorded. Generally speaking, there is no longer disagreement as to the scientific importance of kidney biopsies; however, the practical clinical value of kidney biopsy is still debated, and conflicting points of view persist.

In what follows, a survey will be made of the various problems associated with percutaneous kidney biopsy, particularly with regard to technic and complications, and the significance of the method for the diagnosis, prognosis and treatment of the nephropathies. Next, the pathophysiologic importance of the method will be briefly touched upon and an attempt made to establish the indications for kidney biopsy. Finally the more important problems which this field presents for the future will be considered insofar as is now possible.

The History of Kidney Biopsy. Although we shall here be engaged mainly with percutaneous kidney biopsy it should be mentioned that before this procedure was described kidney biopsy during operation had been carried out. As far as we are aware, Jungmann [*46*] was the first to carry out such surgical biopsies in 1924. However, it was chiefly Castleman and Smithwick [*19*], Heptinstall [*36*], and Saltz, Sommers and Smithwick [*100*] who made use of kidney biopsies, obtained during the course of sympathectomy, to throw light on the renal aspects of essential hypertension, particularly the important question of the sequence of hypertension and nephrosclerosis in essential hypertension.

The first to publish findings in percutaneous kidney biopsies were Perez Ara [*85*], and Iversen and Brun [*42*]. The eight biopsies reported by Ara and the eighty biopsies by Iversen and Brun appeared to indicate that it was possible to obtain sufficient kidney tissue for histologic examination without exposing the patient to any serious risk, and that the means was thereby provided to throw new light on the natural history of many nephropathies.

Ara at first performed the biopsy procedure with the patient in the lateral position, on the operating table, using the maximum degree of kidney elevation. Later he preferred to use the prone position, on a sandbag. The kidney could be precisely localized with the aid of a coarse metal net placed on the skin and roentgenologic control. In order to determine the precise depth of the kidney Ara made use of the movements of the biopsy cannula, exploiting his observation that the outer end of the needle moved synchronously with the respiratory movements of the diaphragm when the point of the needle had penetrated the renal capsule. Ara aspirated the biopsy specimen, using either an ordinary cannula (with 1 mm. bore) and a syringe or a special "nephrobiotome" (modified liver biopsy cannula according to Herrera-Pardo).

Iversen and Brun carried out the biopsy with the patient in the sitting position or in the lateral position. They used an Iversen-Roholm cannula with a 1.5 mm. bore [*41*]. Excision and removal of the biopsy specimen were effected by means of the negative pressure produced by a special syringe, in which the piston could be fixed in any desired position.

Di Gaddo, Torsoli and Righini [*24*], and Fiaschi, Ercoli and Torsoli [*29*] took up the method and carried out ten biopsies using the Vim-Silvermann cannula. At the same time they used perirenal air insufflation and roentgenography with the patient in the prone position. These authors also employed the movements of the needle with respiration as a guide to the localization of the kidney. In their opinion this technic was an improvement both with respect to localization of the kidney and evaluation of the histologic preparation.

In 1953 Payet, Pene, Camain, Gouaze and Calvez [*84*] reported their experience with fifty-five aspiration biopsies with the patient in

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the prone position and with a sandbag under the abdomen. The authors concluded that the risk was slight and that in the right hands the method could provide valuable information.

In 1954 Kark and Muehrcke [*47*] published their technic, with which they have carried out a long series of valuable investigations, contributing to the successful introduction of the method of kidney biopsy in English-speaking countries. Kark and Muehrcke also place the patient in the prone position and localize the kidney by means of an exploring needle, observing its movements with respiration. The biopsy is carried out using Franklin's modification of the Vim-Silvermann cannula.

In 1956 Lusted, Mortimore and Hopper [*60*] suggested carrying out the kidney biopsy under constant fluoroscopic observation of the kidney, using an amplifying fluoroscope and injection of contrast medium (50 per cent hypaque®). The authors, who use the Vim-Silvermann cannula, maintain that the method is an advance so far as the safety of the patient and the effectiveness of the method are concerned, but they have not published any material supporting this claim. This method would hardly be suited to biopsies on kidneys with impaired function.

In 1957 Löfgren and Snellman [*62,105*] suggested that those who do not regularly perform kidney biopsies should proceed as follows: Under anesthesia a small incision is made at the tip of the twelfth rib, after which the finger is introduced and blunt dissection is carried out to the left kidney. Guided by the finger the cannula (a Gillman and Gillman syringe) is introduced into the kidney tissue. By this method tissue specimens were obtained in all fifteen cases examined.

The technic of kidney biopsy which we have elaborated at the Kommunehospital will be briefly described: The patient is placed in the prone position with a sandbag under the abdomen. As a rule we perform the biopsy on the right kidney; in a few cases, however, we do it on the left kidney, but consider this the more dangerous because of the spleen and the large vessels. We use an exploring needle but, unlike Kark and Muehrcke, we aspirate the cut cylinder of kidney tissue, using the Iversen-Roholm cannula and syringe for this purpose [*47*].

It is still an open question which of the at least seven cannulas available is preferable, since comparative investigations with respect to their respective effectiveness and possible complications are not available.

Precautions Prior to Kidney Biopsy. As kidney biopsy is contraindicated in hemorrhagic diathesis, the bleeding time, clotting time, thrombocyte count and prothrombin percentage are always determined prior to the biopsy. It is advisable to know the blood group of the patient and to have compatible blood ready in the blood bank. By way of precaution it is advisable to carry out kidney biopsy on hospitalized patients only; in the opinion of some, only those departments particularly interested, and in the position to acquire a reasonable experience, should take up this procedure. We are inclined to subscribe to this point of view.

In a few cases we have performed kidney biopsy on children for diagnostic reasons, but always under anesthesia; Kark et al. have informed us that they carry out kidney biopsy on children without anesthesia.

As kidney biopsy, in our view, has great diagnostic significance in acute renal failure, and is therefore of significance as a diagnostic aid prior to hemodialysis, it seemed worthwhile to determine the risk attached to kidney biopsy when carried out prior to this treatment, during which, of course, the patient must be given heparin. Throughout the period from January 1954 to January 1958 seventy-four kidney biopsies have been attempted in our hemodialysis unit; nineteen of these biopsies were carried out from one to six days prior to the hemodialysis. In eighteen patients there were no complications on taking the biopsy specimen, or in connection with the subsequent hemodialysis. In one patient (acute anuria), hemodialysis had to be instituted twenty-four hours after the kidney biopsy because of the sudden onset of unexpected hyperpotassemia; after a few hours of dialysis profuse urethral bleeding occurred owing to a fistula which had developed between the renal pelvis and the renal artery as a result of the biopsy. Nephrectomy was necessary; the patient recovered. We are of the opinion that kidney biopsy prior to hemodialysis is valuable and justifiable, but that it is advisable to wait two or three days after a biopsy before subjecting the patient to hemodialysis.

Prior to kidney biopsy, we have at various times (when renal function permitted) carried out intravenous urography, both with and without a radiopaque marker placed on the skin for the purpose of locating the kidney; for a time we also took ordinary roentgenograms of the kidney with a thumb tack placed on the skin,

and in some few cases retrograde pyelography was also carried out as an aid to localization. It is our impression that on the whole these various roentgenologic controls do not aid much in locating the kidney, at any rate not in comparison with the advance introduced by the use of the exploring needle.

Precautions Following Kidney Biopsy. A patient on whom kidney biopsy has been performed should remain in bed during the first twenty-four hours following the procedure and, if there is any suspicion of bleeding, observation of the patient must be intensified, particularly with respect to pulse, blood pressure and the appearance of the urine. All urine passed during the twenty-four hours after a biopsy should be collected and examined for gross hematuria and blood clots; in case of gross hematuria the patient should remain in bed until it is certain that the bleeding has stopped.

Muehrcke, Kark and Pirani [*68*] advise leaving the patient in the prone position on the sandbag for at least the first thirty minutes after the biopsy, which perhaps assists hemostasis, provided the patient can tolerate the prone position. In our experience a fall in blood pressure may occur in a few patients as a result of the prone position, presumably because of a reduction in the venous return to the heart. The prone position is not particularly suitable for patients with cardiac and pulmonary disease who suffer from dyspnea.

Contraindications. Various authors have enumerated a rather long list of diseases to be considered as contraindications to kidney biopsy. There is complete agreement that hemorrhagic diathesis is a contraindication, and in this connection it must be repeated that kidney biopsy should not be performed immediately prior to the administration of heparin as for example in hemodialysis. A further condition is that the patient must be tranquil; kidney biopsy should be avoided in agitated patients. The biopsy may be carried out satisfactorily in unconscious patients provided the airway is kept free. Finally, it is agreed that kidney biopsy should not be performed when it is known that the patient has only one kidney.

A number of authors (Lich [*57*], Lusted, Mortimore and Hopper [*60*], Muehrcke, Kark and Pirani [*67*] and Zelman [*107*]) have maintained that uremia must be considered a contraindication because of the hemorrhagic diathesis which may accompany the condition. We have not regarded uremia *per se* a contraindication to renal biopsy, and do not believe that a hemorrhagic diathesis is so frequent in uremia as to contraindicate kidney biopsy on such grounds in all cases of uremia. To throw further light on this question, we have compared the frequency of gross hematuria following kidney biopsy in patients with and without uremia. Biopsy has been carried out 119 times in patients with uremia (here defined as serum creatinine $\geqq$ 6.0 mg./100 ml.). Of these 119 cases, eighty-eight represented acute uremia and thirty-one chronic uremia. Gross hematuria on the day of biopsy was found in twelve (10.1 per cent); hematurias persisting after the day of biopsy occurred in ten (8.4 per cent). In 365 biopsies carried out on patients without uremia, the corresponding figures were twenty-eight (7.7 per cent) and twenty-three (6.3 per cent). The frequency of hematuria in patients with uremia does not show a statistically significant deviation from the frequency of hematuria in the other patients. In our material, therefore, there is no basis for the assumption that biopsy in cases of uremia carries any special risk. On the contrary, we are of the opinion that uremia of unknown etiology should be considered an indication for kidney biopsy, at least when the uremia must be presumed to be acute.

There is agreement that pyelonephritis cannot be considered a contraindication (Muehrcke, Kark and Pirani [*67,68*], Lusted, Mortimore and Hopper [*60*]). We have examined the frequency of temperature rise after kidney biopsy, paying particular attention to chronic pyelonephritis, in order to determine whether or not the procedure might activate the inflammation. In sixty-two kidney biopsies carried out on patients with chronic pyelonephritis, temperature rises to above 38°C. were observed in 2 (3.2 per cent), while in the remaining group of 422 cases, comprising all kidney diseases with the exception of pyelonephritis, temperature rises were observed in fifteen (3.6 per cent). A rise in temperature is thus a very rare sequel to kidney biopsy. There is nothing to suggest that pyelonephritis is activated by a renal biopsy.

We have no experience with tuberculosis of the kidney but doubt that kidney biopsy should involve any great risk. The same may be said of tumours of the kidney. Nor are hydronephrosis, pyonephrosis and perirenal abscess contraindications in our opinion.

The Effectiveness of Various Kidney Biopsy Meth-

ods. The frequency with which various authors have obtained kidney tissue adequate for diagnosis has been reported as follows: Fiaschi, Ercoli and Torsoli [*29*] obtained sufficient tissue in 60 per cent, Parrish and Howe [*79*] in 78 per cent, Payet, Pene, Camain, Gouaze and Calvez [*84*] in 64 per cent, Reubi [*94*] in 75 per cent, Howe [*37*] in 69 per cent, Muehrcke, Kark and Pirani [*67,68*] in 96 per cent, Leonardi and Ruol [*55,56*] in 58 per cent, De Wardener and Hutt [*23*] in 75 per cent, Lusted, Mortimore and Hopper [*60*] in 90 per cent, Seltzer [*103*] in 47 per cent, Schwiebinger and Hodges [*102*] in 59 per cent, Bucht [*16*] in 72 per cent and Ross and Ross [*95*] in 88 per cent; Löfgren and Snellman [*62,105*] proceed as already described with very great efficiency (100 per cent), which of course is not surprising.

In 1958 Brun and Raaschou [*15*] reported their experience from Poul Iversen's department at the Kommunehospital. From May 1949 to July 1954 biopsy was carried out with the patient in the sitting position; 267 attempts at kidney biopsy were made by this method, and 106 successful biopsies were achieved (40 per cent). Since July 1954 the patient has been placed in the prone position with a sandbag under the abdomen; up to January 1958 a further 243 biopsies have been carried out, with positive results in 162 cases (67 per cent). In each method, the effectiveness increased with practice.

Complications in Kidney Biopsy. The literature contains descriptions of certain complications following kidney biopsy which are deserving of further mention. An account will be given also of the complications in two more extensive biopsy series.

In 1952 Alwall [*1*] reported that in 1944 he had had one death after kidney biopsy in a series of thirteen biopsies. This occurred in a patient who went into shock after the biopsy puncture, and who apparently was first treated twelve hours later. No hematoma could be demonstrated at autopsy, which must raise serious doubts as to whether or not the death should actually be ascribed to the kidney biopsy. The author, in a later publication (1955) with Erlanson and Tornberg [2] has in fact considered the possibility that the death might have been due to retrograde pyelography which was carried out at the same time. We share Alwall's latter opinion.

Among fifty biopsy attempts, Parrish and Howe [*78*] have had one serious complication: kidney biopsy performed with a Turkel biopsy instrument evoked a large retroperitoneal hematoma in a patient with hypertension. The hematoma was located, drained and packed, and the patient made an uneventful recovery. Howe [*37*] stated later that surgical intervention possibly was unnecessary in this case.

Reubi [*94*], in a series of biopsies (number not stated), saw one death ascribed to kidney biopsy. Autopsy provided no explanation for the death. The site of the biopsy could hardly be seen, the hilum vessels and pelvis were not traumatized, and both renal pelves contained urine. Potassium intoxication might possibly have been the immediate cause of death, but the article contains no data on this point.

Ross and Ross [*95*] observed one patient in whom a large retroperitoneal hematoma developed after kidney biopsy, necessitating blood transfusion. The patient had malignant hypertension with uremia. The biopsy specimen showed severe amyloid infiltration of the glomeruli and blood vessels, including a large artery from which the bleeding had probably occurred; there was no hematuria. The patient was discharged symptom-free fourteen days after the biopsy.

Zelman [*107*] reported a case of bleeding after simultaneous liver and kidney biopsy in a moribund patient suffering from severe acute glomerulonephritis with uremia following an upper respiratory infection. After the biopsy specimens were obtained the patient gradually lapsed into coma and died about ten hours later. Hemorrhage was not suspected but at autopsy the abdominal cavity was found to be filled with 2 L. of fluid and clotted blood. Right perirenal hemorrhagic infiltration was present, amounting to about 0.5 L. of blood. Hemorrhages were found subendocardially, in the stomach mucosa and in the renal pelves. The autopsy report suggested the diagnosis of acute interstitial nephritis or a pyemic kidney. The author concluded that the bleeding in this case may have been caused by increased capillary fragility, which may have been present prior to the biopsy as a result of the uremia.

Muehrcke, Kark and Pirani [*67,68*] in 1955 reported the complications encountered by them in 100 kidney biopsy attempts in eighty-seven patients, kidney tissue being obtained in ninety-six instances. Microscopic hematuria was observed in all ninety-six instances. The only complications noted were gross hematuria in seven cases and loin pain in eight.

Finally, Brun and Raaschou in 1958 [*15*] summed up their experience with regard to complications in kidney biopsy material consisting of 510 kidney biopsy attempts in 406 patients. The complications of major importance observed were gross hematuria on the day of biopsy in 7.9 per cent and hematuria persisting after the day of biopsy in 6.6 per cent. Blood clots were observed in the urine nineteen times in the 510 attempts, and ureteral colic due to passage of clots, five times. The frequency of gross hematuria after biopsy in patients with arterial hypertension and in patients with uremia did not deviate significantly from the frequency of hematuria in the remaining patients. Shock following kidney biopsy was observed twice; both patients recovered. A blood transfusion following kidney biopsy attempts was given in eleven cases (2.1 per cent). The twenty-four-hour endogenous creatinine clearance was examined both prior to and after the biopsy in 173 kidney biopsy attempts. In only two cases, a major transient decline in kidney function was observed after the biopsy, due in both instances to blockage by clots; on removal of the clots the creatinine clearance quickly returned to the initial value. In the remaining cases the creatinine clearance either rose or was unchanged after biopsy. A moderate retroperitoneal hematoma was noted in one case but surgical intervention was not considered necessary. A minor lesion of the gallbladder was observed in a single patient. Seven per cent of the patients complained of lumbar pain after the kidney biopsy, usually of slight or moderate degree. No deaths occurred as a result of kidney biopsy in this series. It is concluded that the risk involved in kidney biopsy is slight.

How Representative Is the Tissue Obtained in Kidney Biopsies? Muehrcke, Kark and Pirani [*67*] have examined this question by taking samples with the biopsy cannula from ten different points of the kidney in a number of kidneys at the autopsy table; these biopsy specimens were compared with one another and with larger wedge sections taken from the same points in the kidney. There was excellent correlation between the ten biopsies and the larger wedge sections of the kidney. Muehrcke et al. consider a kidney biopsy specimen acceptable when it contains at least 5 glomeruli, at least in the case of diffuse nephropathies. Sala [*99*], in 100 cases of nephropathy, compared postmortem kidney biopsies (containing at least 10 glomeruli in each biopsy) with ordinary autopsy material from kidney tissue, and came to the conclusion that in the diffuse nephropathies there was good agreement between the two methods.

Kark et al. [*51*], in a series of twenty-four patients with lupus nephritis and nine patients with disseminated lupus erythematosus and normal kidneys, examined the correlation between three simple kidney function tests (urea clearance, phenolsulfonphthalein excretion and specific gravity concentration) and the degree of renal damage indicated in the kidney biopsies, and found a good correlation. It has also been our experience (at least with the diffuse nephropathies) that there was always good agreement between kidney function and histologic findings [*11*], and there has never been any question of our having been misled by kidney biopsies in regard to the extent of the histologic lesions. While Muehrcke et al. demand at least 5 glomeruli in a biopsy specimen to be able to make a diagnosis, it should be noted that in several nephropathies (for example amyloidosis and diabetic glomerulosclerosis) the diagnosis can be made on fewer glomeruli. It is possible that one could be misled by kidney biopsies in acute and chronic pyelonephritis, due to the focal character of the disease; it is obvious that kidney biopsy can mislead when diseases of the kidney of such focal character as tumor, abscess and tuberculosis are in question.

The Normal Histology of the Kidney, Based on Kidney Biopsies. The published material on the histology of the normal kidney on the basis of kidney biopsies is so scanty that secure conclusions cannot be drawn. This is true, for example, in regard to the question as to how far the appearance of the tubule cells can be correlated with their function. Bjørneboe, Brun, Gormsen, Iversen and Raaschou [*7*] made the observation that glomeruli, even in normal kidney biopsies, contain surprisingly few erythrocytes when the tissue is fixed in alcohol; Muehrcke, Kark and Pirani [*67*] are of the opinion that this is due to their use of an aspiration technic. The reason actually is that Muehrcke et al. use formalin as a fixative, since by using this fixative we also find erythrocytes in the glomerular capillaries of kidney tissue obtained by the aspiration technic.

The Significance of Kidney Biopsy for Diagnosis and Prognosis. Acute anuric renal failure: In the case of acute renal failure, kidney biopsy has shown itself to be of considerable value [*12,13,18,33,42,43,80*]. We consider it an

important aid in the selection of patients for treatment by hemodialysis, because otherwise one is often unable to decide whether a patient with anuria has a reversible form of acute renal failure (for example, shock kidney), an irreversible form of acute kidney disease, or is in the terminal stage of a chronic kidney disease. Should the biopsy show that the glomeruli are completely or almost completely destroyed by acute or chronic lesions then, with the therapeutic resources we command today, there is no possibility of recovery. In such cases hemodialysis is seldom indicated, and if it were, one would hardly dialyse repeatedly over longer periods.

Tubular lesions (ischemic, toxic) are reversible to a much greater degree than glomerular lesions, and we believe that should tubular lesions be demonstrated in cases of acute renal failure, treatment by hemodialysis should be repeated as frequently as required, possibly "to the bitter end."

Renal biopsy has also made it possible to examine the histologic picture in non-lethal cases of acute anuric renal failure, and to follow the pathologic lesions throughout the course of the disease.

In the examination of kidney biopsy specimens from patients with acute renal failure following shock, there is often a striking and inexplicable disproportion between the few and slight lesions that can be demonstrated by the classic histologic technic and light microscopy, and the severe disturbances in kidney function accompanying this disease picture (Iversen and Brun [*42*], Brun [*12*], Brun and Munck [*13*]). The results of electron microscopic and histochemical investigations on kidneys from such patients will be awaited with great interest.

It has been shown that the occurrence of frank tubular necrosis is infrequent even in severe cases of anuria, and that interstitial inflammatory infiltration and interstitial edema are among the more common findings. Hydropic changes in the epithelium of the proximal tubules have been found in many cases, but it is probable that these changes were due to infusions of dextran, sucrose, sulphate, etc., and not to the kidney disease as such (Brun and Munck [*13*]).

Lassen and Thomsen [*54*], in a study of twenty-eight patients with so-called hepatorenal syndrome, found that the histologic picture of the kidney lesions, as seen in biopsies, did not differ from that encountered in acute renal failure following shock. By a thorough study of the case histories they could, in nearly all cases, find evidence of a period of shock preceding the renal shutdown. They concluded that the hepatorenal syndrome is nothing more than ischemic anuria in patients with liver or biliary disease.

Kidney biopsies were performed by Casey and Woolf [*18*] in two cases of toxic anuria caused by organic solvents. One was a patient who on admission was thought to have enteritis or obstruction of the small intestine. A biopsy performed on the first day of oliguria contributed substantially to the diagnosis; it revealed pronounced changes, mainly confined to the proximal tubules, which showed dilatation and focal sloughing of the epithelium. A second biopsy was performed during the phase of polyuria, but the kidney did not appear much changed. A similar lack of correlation between the histologic picture and urine output and duration of illness has been found in acute renal failure following shock (Brun and Munck [*13*]).

The nephrotic syndrome: The nephrotic syndrome is one of the groups of diseases for which kidney biopsy offers the greatest diagnostic support, since on the basis of the clinical criteria alone one often cannot distinguish with sufficient certainty between the various underlying diseases. Moreover, as stressed by Pollak, Kark, Pirani, Soothill and Muehrcke [*91*], kidney biopsy has become of importance in evaluating prognosis in the nephrotic syndrome. Whereas previously it was difficult to make a prognosis on the basis of the clinical data, it now appears that the patient's prospects can be better judged by taking into account the extent and degree of severity of the glomerular damage.

The diagnostic value of kidney biopsy in the nephrotic syndrome was shown by Bjørneboe, Brun, Gormsen, Iversen and Raaschou [*7*]: biopsy in eight cases of this syndrome showed that no less than four patients had amyloidosis of the kidneys; the other four had chronic glomerulonephritis. Two had hardly any glomerular changes, and were perhaps cases of so-called "genuine lipoid nephrosis" (diabetic nephropathy had been excluded). Kidney biopsy was of diagnostic importance especially in the cases of amyloidosis of the kidneys, in which the underlying disorder did not manifest itself distinctly in the clinical picture.

These observations were later confirmed in more extensive studies by Howe [*37*], and Pollak, Kark, Pirani, Soothill and Muehrcke [*91*], as well as in a smaller series by Ross and Ross [*95*].

In Pollak et al.'s study, comprising fifty-six adult patients with the nephrotic syndrome, it is pointed out that the most frequent causes are: subacute glomerulonephritis, diabetic glomerulosclerosis and lupus nephritis (lupus erythematosus disseminatus); one is rather surprised to find only one case of amyloidosis in this extensive case material. On the other hand, Howe [*37*], in a series consisting of twenty-two cases of the nephrotic syndrome, comes to the conclusion that chronic glomerulonephritis (type II nephritis) and diabetic nephropathy are the most frequent causes of the syndrome.

In the nephrotic syndrome, serial kidney biopsies are particularly suited to elucidating the progression of the underlying diseases from their earliest stages, in which insignificant glomerular lesions are found (Kark, Muehrcke, Pirani and Pollak [*50*], Ross and Ross [*95*]). Muehrcke, Kark, Pirani and Schoenberger [*66*] have found that, as seen in light microscopy, the earliest morphological lesion in the nephrotic syndrome with subacute glomerulonephritis is moderate to severe fatty degeneration of the tubular cells, with normal glomeruli; thickening of the basement membranes and increased content of cells; crescents in the glomeruli are not observed till later.

Farquhar, Vernier and Good [*27,28*] studied kidney biopsy specimens, obtained from sixteen patients with the nephrotic syndrome, by means of the electron microscope. In every instance a striking abnormality was observed, consisting of a loss of the characteristic organization of epithelial cytoplasm into foot processes. Instead, broad masses of epithelial cytoplasm were seen partially or completely to cover the surfaces of the capillary loops. Additional changes were observed in the epithelial cytoplasm and in the endothelium, which often appeared swollen. The basement membrane was somewhat thicker than normal. The changes differed strikingly from those seen in glomerulonephritis and lupus nephritis.

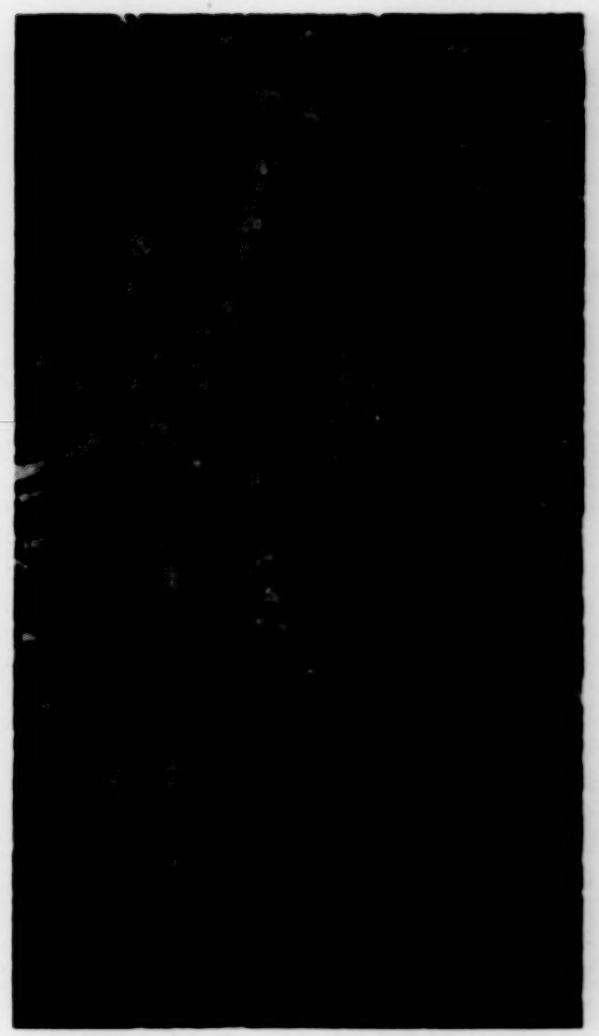
Dalgaard, of our group, has also demonstrated loss of the foot process organization in a seventeen year old boy with a nephrotic syndrome of three weeks' duration, a normal twenty-four-hour endogenous creatinine clearance, and a urinary protein excretion of 10 to 20 gm. per twenty-four hours. (Figs. 1, 2 and 3.)

Pollak et al. [*88,89*] obtained serial kidney biopsy specimens in a case of bilateral thrombosis of the renal veins with the nephrotic syndrome. The interstitial tissue showed pronounced edema, and there was focal or diffuse thickening of the basement membrane in the glomerular capillaries, together with thickening of Bowman's capsule; the tubules showed atrophy and dilatation and frequently contained casts and protein-containing material. The findings seem to be rather unspecific.

Acute glomerulonephritis: Although kidney biopsy specimens from patients with "acute glomerulonephritis" have been studied by a number of investigators, the method has thus far not altogether clarified the question as to just what this term actually includes. This designation is employed in some cases [*75*] to describe a more or less well defined clinical picture in which a streptococcal infection, after a course of one to two weeks, is followed by hypertension, edema, proteinuria and hematuria, and to which there correspond histologic lesions consisting of an intense proliferation and inflammation of the glomerular tufts. At times, similar symptoms and histologic lesions are produced by other causes, for example, polyarteritis nodosa, systemic lupus erythematosus and allergic diseases. Should the term acute nephritis be employed in these cases too?

Bates, Jennings and Earle [*3*] have reported ten cases of a disease in which hematuria was associated with acute pharyngitis, and clinical and immunological evidence ruled out recent group A hemolytic streptococcal infections. In nine cases, in which kidney biopsies were obtained while the hematuria was still present, glomerular damage was found which was much milder and presumably more focal in distribution than the more severe and proliferative changes observed in patients in whom acute nephritis followed a group A hemolytic streptococcal infection. Should this acute kidney disease also be called acute nephritis?

Other investigators [*14,34,37,38,40,91*] have carried out biopsies on patients with clinical acute nephritis, but the results achieved can still not be said to have provided any clarification of the state of affairs in this disease group. As far as the gravity of the disease is concerned, there appears to be a good correlation between the clinical picture and the histological findings; on the other hand, estimation of the duration of the disease from the histologic criteria appears to be less certain, presumably because of ignorance of the histologic lesions in the earlier phases.



1



2

FIG. 1. Changes in the epithelium of a typical glomerulus. Epithelial cytoplasm covers the luminal side of the basement membrane completely. No regular organization into foot processes is seen. × 14,500.

FIG. 2. Greatly thickened and distorted basement membrane-like material is seen between two capillaries. Note the complete lack of foot processes. × 14,500.

Farquhar, Vernier and Good [*27,28*] observed in electron microscopic studies that the glomerular changes in acute nephritis differed substantially from the changes seen in lupus nephritis and the nephrotic syndrome. In acute nephritis the glomeruli showed predominantly proliferative changes in the endothelium and basement membrane. Hypercellularity was primarily due to an increase in the number of endothelial cells, secondarily to proliferation of epithelial cells and intraluminal accumulation of mononuclear cells. In spite of the epithelial swelling, the epithelial foot process organization usually appeared normal except in areas of severe damage. Accumulations of material resembling the basement membrane were seen and, especially in the more damaged glomeruli, large masses of newly formed basement membrane-like material were present in bars or irregularly shaped strands interlaced with endothelial cytoplasm. It was suggested that the basement membrane-like material is synthesized as a result of endothelial activity.

There is little doubt that the electron microscopist can find changes in glomeruli which appear normal in light microscopy.

It is likely that continued meticulous studies of serial biopsies, correlated with comprehensive clinical observations, will give clues to a better understanding of the natural history of the group

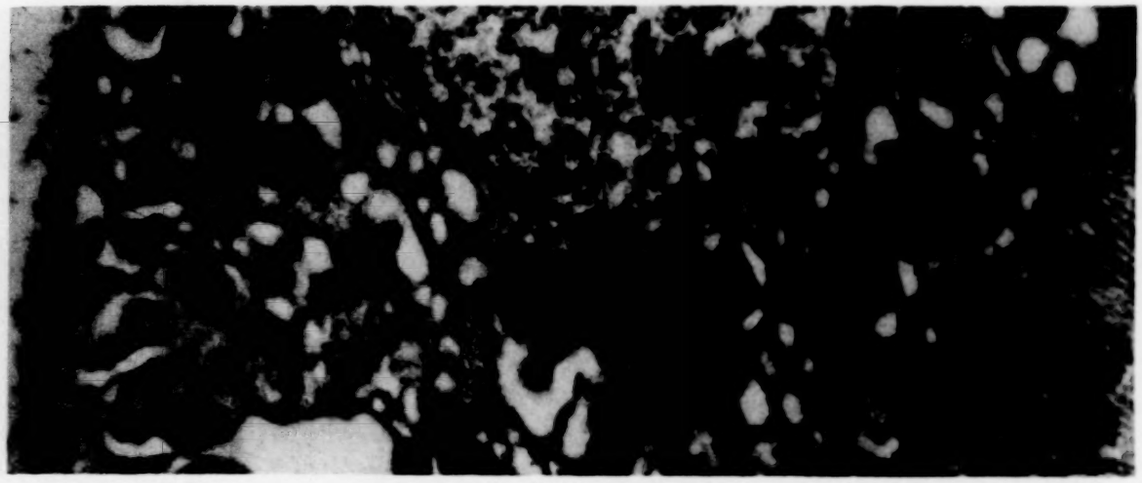


FIG. 3. Part of a proximal convoluted tubule cell. The basement membrane is seen to the extreme left. An elaborate system of membranes divides the basal cytoplasm into compartments with mitochondria, large vesicles and groups of small granules. Large granules or droplets are also seen. They are derived from mitochondria as they show occasional remnants of their internal membranes. At the right a well conserved brush border is seen. × 14,500.

of diseases which, for lack of better knowledge, we call nephritis.

Lupus nephritis: A number of investigators have examined the histological lesions in the kidney by means of kidney biopsy. In 1954 Lister and Baker [*58*] reported a case in which the diagnosis of systemic lupus erythematosus (SLE) could be made after examining a kidney biopsy specimen; a series of other examinations in this patient did not lead to any certain diagnosis.

Muehrcke, Kark, Pirani and Pollak [*73*] in 1957 published a comprehensive monograph on lupus nephritis (LN), based on examination of thirty-three patients with SLE, renal biopsy having been carried out once or several times (in all, fifty-seven biopsies). Twenty-four of these thirty-three patients had renal involvement on histologic evidence (73 per cent). The time of appearance of renal involvement in SLE was variable. The authors conclude that this may occur at the onset of the disease or at any time thereafter until just before death.

Twelve patients had severe kidney lesions when first seen and these were classified as glomerulonephritis. Progression to chronic glomerulonephritis and contraction of the kidney was seen in only four cases. Seven patients had the nephrotic syndrome at some time during the period of study and three had what the authors call a "pseudo-nephrotic" syndrome, i.e., all criteria of the nephrotic syndrome were satisfied except that the serum cholesterol was low or normal. A low cholesterol nephrotic syndrome implied a grave prognosis and rapid death. The most important histologic changes in the glomeruli were: (1) wire loop lesions, (2) fibrinoid changes, (3) hyaline thrombi, (4) necrotic lesions, and (5) hematoxylin bodies. The tubular lesions seemed to lack specificity and were difficult to interpret. With the exception of "hematoxylin bodies," no single histological feature could be considered pathognomonic of LN, but wire loops, local necrosis, fibrinoid and hematoxylin bodies occurred frequently in a combination peculiar to lupus nephritis.

With respect to the effect of treatment with corticotropin and cortisone on the progression of renal lesions, the authors concluded that they did not find any evidence of regression of structural damage to the kidney. However, they found no evidence that treatment with hormones caused progression.

Joske and Stubbe [*45*] also examined kidney biopsy specimens from a number of patients with SLE. Nine of eleven biopsy specimens showed abnormalities, gross changes being present in five. They concluded that renal biopsy may be of value in SLE, partly as a tool in investigation and partly in prognosis, by detecting otherwise inapparent renal manifestations of this disease.

Farquhar, Vernier and Good [*27,28*], by electron microscopic examination of kidney biopsy material from three patients with SLE, found the most characteristic glomerular lesion to be a generalized increase in thickness of the basement membrane (from 4 to 6 times the normal thickness) associated with a variable degree of endo-

thelial proliferation. The epithelium did not appear to be altered until basement membrane thickening was quite severe. In one case "fibrinoid" appeared to accumulate between the basement membrane and the endothelium.

Diabetic nephropathy: Kidney biopsy has shown itself to be a valuable tool in the study and diagnosis of late diabetic kidney disease. Brun, Gormsen, Hilden, Iversen and Raaschou [*11*] in 1953 indicated the usefulness of kidney biopsy in the early cases which do not yet exhibit distinct clinical signs and symptoms of nephropathy. The study also supported the theory that diabetic nephropathy starts as diffuse hyalinization of the basement membranes in the glomeruli, and that the nodular lesions may be considered to be a further development of the diffuse lesions.

Taft, Finck and Joske [*106*] were particularly interested in comparing the clinical history with the histological findings in diabetic nephropathy. They further pointed to the important fact, that completely normal kidney biopsies may be observed in both young and old diabetic persons who have had the disease from fourteen to twenty-one years.

A particularly interesting investigation has been reported by Darnaud, Denard, Moreau and Suc [*21,22*]. These authors used periodic acid-Schiff staining to demonstrate the glycoproteins which presumably constitute the characteristic deposits in the glomeruli. They believe that they have demonstrated the diffuse nodular lesion to be a further development of the diffuse lesion.

Howe [*37*] emphasized that in diabetic persons with the nephrotic syndrome, kidney biopsy is the only means of deciding whether or not the typical nodular Kimmelstiel-Wilson lesion is present; a number of diabetic subjects with the nephrotic syndrome actually showed only what he calls an "arteriolo-nephrosclerosis," that is, moderate diffuse thickening of the basement membrane in the glomeruli, together with hyaline thickenings of the arterioles.

Electron microscopic studies of diabetic nephropathy are still in their infancy; Bergstrand and Bucht [*6*] have examined one case of diabetic glomerulosis, with the aid of electron microscopy, in order to determine more clearly the exact localization of the precipitates in the glomeruli. They found that the specific glomerular lesions in diabetic glomerulosclerosis consist of a thickening and folding of the capillary basement membrane accompanied by precipitation of a hyaline material in the cytoplasm of the endothelial cells and proliferation of these cells. Changes were also observed in the basement membrane of Bowman's capsule.

Chronic pyelonephritis: There is a single study available, by Kipnis, Jackson, Dallenbach and Schoenberger [*53*], on the significance of kidney biopsy for the diagnosis of chronic pyelonephritis. The rather limited number of observations indicated a good correlation between morphology and function in a disease of such focal nature as pyelonephritis. The authors were the first to have cultivated bacteria from the biopsy specimens, obtaining positive cultures in only two of thirteen patients with infection of the urinary tract.

Kark, Muehrcke, Pirani and Pollak [*49*] have investigated a special type of pyelonephritis: Five patients with hematuria and proteinuria aroused no clinical suspicion of pyelonephritis, as pyuria, pain in the loins, fever, dysuria and bacteriuria were not present. By cultivating bacteria from the kidney biopsy specimens and determining their resistance, the authors succeeded in arriving at a treatment which apparently provided a cure for the disease; they call this kidney disease "subacute, bacterial nephritis."

Various authors agree that pyelonephritis is not activated by the kidney biopsy procedure, an observation supported by our own experience, as previously mentioned.

When evaluating kidney biopsies under circumstances in which the diagnosis of pyelonephritis comes into question, one should bear in mind that a normal biopsy does not exclude this diagnosis, on the one hand because of the focal nature of the disease, on the other because it so often occurs unilaterally.

Arterial hypertension: Essential hypertension and nephrosclerosis have not been the subject of investigation with the aid of percutaneous kidney biopsy, undoubtedly because this disease has been so thoroughly investigated by means of biopsy specimens taken during sympathectomy [*19,26,30,31,36,100*]. As these studies thus lie outside the scope of this review, the results will be only briefly touched upon. In Castleman and Smithwick's investigation it was pointed out that arteriolar lesions are scanty in most cases of essential hypertension, from which the important conclusion was drawn that these scattered vascular lesions cannot be regarded as giving

rise to hypertension on the basis of ischemia, and that they must be considered to be secondary to the hypertension. In 1957 Saltz, Sommers and Smithwick published the findings in a very extensive biopsy material, from 1,251 patients with hypertension who had undergone sympathectomy; they concluded, on the basis of clinicopathological evaluation of a large part of this material, that kidney biopsy should be considered an important supplement to other known methods of evaluating hypertension.

Toxemia of pregnancy: Pollak, Pirani, Kark, Muehrcke, Freda and Nettles [*90*] have carried out serial renal biopsies in ten patients with toxemia of pregnancy. Two distinct lesions were found in the glomeruli of women with this syndrome: Either generalized swelling of the glomerular tuft or thickening of the basement membrane; the latter was associated with vascular changes. These lesions probably are the pathological counterpart of pre-eclampsia and of essential hypertension with nephrosclerosis, respectively. In a third group, lesions of both types were observed and the swelling disappeared postpartum. These could be either cases of essential hypertension with superimposed pre-eclampsia or cases of severe pre-eclampsia producing permanent vascular changes.

Renal sarcoidosis: Kidney involvement is uncommon in sarcoidosis. Löfgren, Snellman and Lindgren [*63*] studied sixteen patients with typical pulmonary sarcoidosis by means of kidney biopsy and renal function tests: in six patients with hypercalcemia kidney function was reduced, and on kidney biopsy specific lesions were found (hyalinization of Bowman's capsule, epithelioid cell granulomas and calcareous deposits); in the remaining cases without hypercalcemia kidney function was only slightly reduced, and as a rule no specific degenerative lesions were found in the kidney tissue. Berger and Relman [*4*] described a case of sarcoidosis localized to the kidneys; this patient did not have hypercalcemia, and the kidney disease did not improve with cortisone treatment.

Multiple myeloma: Greenwald, Bronfin and Auerbach [*35*] have performed kidney biopsy in five patients with myelomatosis and nephropathy; diffuse histologic kidney lesions were seen, but nothing which could be considered specific for myelomatosis.

Bilateral cortical necrosis: This rare disease of the kidney has been diagnosed four times *in vivo* with the aid of kidney biopsy (Gormsen, Iversen and Raaschou [*33*], Gjørup, Killmann and Thaysen [*32*], Boucot, Guild and Merrill [*10*], Ober, Reid, Romney and Merrill [*76*]). In the case reported from our clinic kidney biopsy was decisive in leading to initiation of adequate treatment by peritoneal dialysis, with recovery from prolonged and severe anuria and uremia. The patient's renal function was subsequently gradually restored and a year later reached a maximum value of about one-half the normal; two years after the disease she carried through a normal pregnancy.

Nephrocalcinosis: Kidney biopsy has great importance for the diagnosis of the many different forms and causes of nephrocalcinosis; a study by Bjørneboe, Brun, Gormsen, Iversen and Raaschou [*9*] on nephrocalcinosis in hyperparathyroidism and vitamin D_2 poisoning may be mentioned as an example of this; the prognostic significance of the presence or absence of glomerular damage in this connection was also demonstrated.

Nephropathy of potassium depletion: The recently elaborated clinical concept of the nephropathy of potassium depletion (kaliopenic nephropathy) offers a suitable field for renal biopsy investigations (Conn and Johnson [*20*]). There is much to suggest that chronic hypopotassemia in connection with a significant deficit in the potassium stores of the body can result in characteristic anatomic and functional lesions of the convoluted tubules. Such a nosologic entity does exist but it should be stressed that the histologic findings typical of the disease (tubular vacuolar nephropathy, clear cell nephrosis) are hardly specific. Attention may be drawn to the similar lesions observed by Brun and Munck [*13*] in acute anuria in which hypopotassemia was not present; these lesions were all found in patients who had received dextran.

Relman and Schwarts [*92,93*] obtained kidney biopsy specimens from three patients with severe potassium deficit due to chronic diarrhea and found hydropic and degenerative lesions in the convoluted tubules, but no glomerular or vascular lesions; when the potassium balance was re-established the histologic lesions disappeared completely in one patient and improved in another. Further biopsy studies of this interesting syndrome would be desirable.

Amyloidosis of the kidney: Kidney biopsy has particular diagnostic significance in amyloidosis of the kidney, as this diagnosis is often confused

clinically with other chronic nephropathies and cannot be made in any other way with certainty. Cases of amyloidosis of the kidney diagnosed by this means have been described by Bjørneboe, Brun, Gormsen, Iversen and Raaschou [7], Alwall [1], Ross and Ross [95] and Muehrcke, Kark and Pirani [67]; in the last-named investigation the patient had amyloidosis in spite of a negative Congo red test.

In primary amyloidosis, kidney biopsy is of particular importance in diagnosis, because of the absence of other diseases known to cause amyloidosis. So far two cases have been published of this disease in which kidney biopsy has been of value in establishing the diagnosis (Bjørneboe, Brun, Gormsen, Iversen and Raaschou [7], Muehrcke, Pirani, Pollak and Kark [69]).

The Significance of Kidney Biopsy in Therapy. While the significance of kidney biopsy for investigation of renal diseases is indisputable, opinions are divided as to its practical clinical value. While Kark and his school, as well as ourselves, consider it important in the diagnosis and prognosis of renal diseases, others view its significance in this respect as more limited (Bucht [16], Ross and Ross [95]). It is true that at the moment its significance in the treatment of renal diseases is limited if only because our present therapeutic means are so restricted. Nevertheless, areas are now appearing in which one might justly maintain that benefit may be derived from the use of kidney biopsy as a guide in the treatment of renal diseases. In the following section some examples of this will be briefly mentioned.

Extra-corporeal dialysis: We often perform kidney biopsy at the Kommunehospital in patients with acute renal insufficiency, for one thing with a view to the differential diagnosis between acute renal failure and chronic nephropathy with anuria. We consider the exact histologic diagnosis to be of importance in evaluating how actively the patient should be treated by hemodialysis. As previously mentioned, we wait from two to three days after the biopsy before carrying out the dialysis.

Kidney biopsy is employed to a much lesser extent at a number of other dialysis centers. For example, Merrill [65] is very cautious in his use of kidney biopsy due to fear of hemorrhage in a possible subsequent dialysis, in view of the heparinization necessarily involved. Merrill first carries out kidney biopsy after two weeks of anuria, and then waits from five to six days before the next dialysis.

In severe acute glomerulonephritis with anuria (Brun, Gormsen, Hilden, Iversen and Raaschou [14]), dialysis is presumably useless when the biopsy specimens show severe destructive glomerular lesions; however, we have not yet had sufficient experience with such patients who have undergone repeated hemodialyses.

ACTH and cortisone treatment in the nephrotic syndrome: Kidney biopsy is a useful aid in the treatment of the nephrotic syndrome; for one thing, it makes possible selection of those patients with the maximum chance of response to ACTH and cortisone, and for another the effect of the hormone treatment on the renal parenchyma can be followed. In 1952, Bjørneboe, Brun, Gormsen, Iversen and Raaschou [8], on the basis of a small number of patients, were able to submit the hypothesis that ACTH has a beneficial effect in patients with sparse glomerular lesions; the correctness of this hypothesis is supported by the work of Ross and Ross [95] on prednisone treatment of type II nephritis and the work of Pollak, Kark, Pirani, Soothill and Muehrcke [91] on ACTH and cortisone treatment of the nephrotic syndrome. Bjørneboe et al. [8], in a patient with the nephrotic syndrome due to "genuine" nephrosis, made the further observation that an originally low tubular epithelium became high after treatment with ACTH. Kark, Muehrcke, Pirani and Pollak [49] made the same observation on a boy with the nephrotic syndrome.

Subacute bacterial nephritis: Reference should be made to page 685 for the significance of kidney biopsy in the treatment of this disease entity, as indicated by Kark, Muehrcke, Pirani and Pollak [49].

ACTH and cortisone treatment in lupus nephritis: As already mentioned, Muehrcke, Kark, Pirani and Pollak [73] found that treatment with corticotrophin and cortisone had no effect on the progression of renal lesions in lupus nephritis. The treatment itself did not cause any appreciable kidney damage.

Finally, it should be mentioned that kidney biopsy is considered of value in cases of diabetes when hypophysectomy or adrenalectomy is under consideration.

The Significance of Kidney Biopsy as a Scientific Tool. Kidney biopsy will probably find its greatest value in this field. Here, interest is first and foremost being directed to the cyto-

chemical and electron microscopic investigations of normal and pathologic kidney tissue. Research in both these fields is only beginning.

As yet, there is very little published on the cytochemical investigations of kidney biopsy samples. For example, Bjørneboe, Brun, Gormsen, Iversen and Raaschou [7] have tried to use Gomori's method for determination of the presence of alkaline phosphatase in the proximal tubules in normal kidney tissue and in the nephrotic syndrome. No results of particular value were obtained in these investigations. Kark, in a personal communication, has informed us that an extensive experimental study is now being carried out on the enzymatic pattern of the renal parenchyma, partly in kidney biopsy sections, partly with microdissected nephrons, but no reports have been published as yet.

The electron microscopy of kidney biopsies constitutes a new field with unlimited possibilities. Studies on the appearance of the glomerular membrane in diabetic nephropathy, nephrosis and acute anuria come to mind. Investigation of the fine structure of the tubule cells in various renal diseases is awaited with the keenest interest. Those interested should refer to the publications of Farquhar, Vernier and Good [*27,28*] and Mueller, Mason and Stout [*74*].

In this connection, the significance of kidney biopsy for an understanding of the mechanism of proteinuria and hematuria should be mentioned. In sections of biopsy specimens from a series of patients with protein-free urine, proteinaceous material was observed both in Bowman's capsule and in the lumen of the tubule [*42,50,66*]. This observation, the correctness of which we can confirm, supports the concept that, to a certain extent, protein is normally filtered by the glomeruli and reabsorbed in the tubules. The same authors believe that in a few cases they have observed the occurrence of hematuria by the rupture of a peritubular capillary and hemorrhage into the lumen of the tubule.

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ADDENDUM

Since the completion of this manuscript our attention has been drawn to the following papers:

(1) Folli, G., Pollak, V. E., Reid, R. T., Pirani, C. L. and Kark, R. M. Electronmicroscopic studies of renal biopsies taken from nephrotic patients before and after diuresis. *J. Lab. & Clin. Med.*, 50: 813, 1957.

(2) Galán, E. and Masó, C. Needle biopsy in children with nephrosis. A study of glomerular damage and effect of adrenal steroids. *Pediatrics*, 20: 610, 1957.

(3) Imura, S. and Shirai, H. A study on the correlation between renal function and histological findings got by renal biopsy. *Rinsho Naika Shonika*, 12: 1079, 1957.

(4) Imura, S. and Shirai, H. Kidney biopsy procedure. *Rinsho to Kenkyu*, 34: 33, 1957.

(5) Jackson, G. G., Poirier, K. P. and Grieble, H. G. Concepts of pyelonephritis: experience with renal biopsies and long-term clinical observations. *Ann. Int. Med.*, 47: 1165, 1957.

(6) Kark, R. M., Muehrcke, R. C., Pollak, V. E., Pirani, C. L. and Kiefer, J. H. An analysis of 500 percutaneous renal biopsies. *Arch. Int. Med.*, 101: 439, 1958.

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On the Diversities of Structure, Perfusion and Function of the Nephron Population*

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THE remarkable advances in renal physiology during the last half century have been made possible by the formulation of a satisfactory theory of urine formation. The demonstration [*1–6*] that glomerular fluid is an ultrafiltrate of plasma banished other hypotheses from the field in supporting the view that urine is produced by the action of tubules upon a glomerular filtrate. On the strength of this finding, clearance methods—based ultimately upon the Fick principle—were devised for the measurement of total glomerular filtration rate and renal blood flow [*7*]; thus making it feasible to define and to assess quantitatively the various discrete tubular functions. Although the kidneys are congeries of organelles—the nephrons—it has become customary to discuss renal function and the localization of individual tubular operations as if one such unit were concerned, or as if dividing total overall function by the number of nephrons would yield a measure of function in the single unit. This simplification would be justifiable if the nephron population were relatively uniform or if each nephron were organized so as to produce urine of same amount and composition each minute. In fact, however, considerable structural variety is clearly demonstrable and much evidence indicates non-uniformities in perfusion, filtration, and tubular behavior. A full understanding of the processes of urine production requires a careful evaluation of these diversities.

Anatomic Evidence of Nephron Diversity. Surprisingly little information is available regarding the nephron population as a whole. Orthodox histologic methodology yields an incomplete and perverted picture of a single nephron, not to mention the entire assembly of units. Unfortunately, dissecting out the individual elements is a tedious and difficult business that, more often than not, is unrewarding. Microdissection involves subjecting the kidney to prolonged treatment with strong hydrochloric acid and careful separation of the adherent, brittle and tenuous tubules, a process in which distortion of shape and dimensions is inevitable. Complete intact nephrons are difficult to obtain and the final figures must suffer some bias in the fact that measurements are obtained only for the tougher and more accessible varieties. For these reasons it is fortunate perhaps that the values in the literature are too few to permit a thorough statistical evaluation. According to Oliver [*8*] only fourteen complete nephrons from human kidneys have been measured [*9,10*]. He has added the dimensions of eighty-three proximal segments (twenty-four, twenty-nine, and thirty from different kidneys) but not of the whole nephrons. Even in the extensive study of mammalian nephrons reported by Sperber [*11*], the number of completely measured nephrons from any single kidney seldom exceed six to twelve. The data [*12–15*] available on glomerular size are also unsatisfactory but owing to the greater ease with which glomeruli may be obtained, they are probably more representative. Taken as a whole, some notion may be obtained regarding the range and character of structural variation among the nephrons, although it must be admitted that generalizations from such small samples are not altogether reliable.

The organization and distribution of the nephrons suggest considerable variability [*8–16*]. Three basic categories, depending upon the site of origin, appear to be present in all mammalian kidneys [*10,11*]. Relatively short nephrons are found in the outermost portion of the renal cortex. They lack a thin limb and are characterized by short loops of Henle which are formed by the ascending or first portion of the

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distal tubule. A second category which arises in the middle and outer zones of the cortex is marked by a short thin limb, the bend of which is also formed by the distal tubule. The longest nephrons begin in the deep cortex, the proximal convoluted tubule coursing irregularly in the vicinity of its glomerulus, and then extending almost directly to the medulla in a ray together with similar segments of other nephrons to form the thin limb which passes a variable and often considerable distance into the papilla. After bending, the thin limb changes gradually into the ascending portion of the distal segment which returns directly to the glomerulus of origin, where it is attached to one of the arterioles before terminating in distal convolutions. The tubules are definitely separated and stacked from the medulla outward in more or less alternating masses of distal and convoluted tubules. The final convergence upon the collecting tubules results in variable distances from the end of the distal segments to the renal pelvis. Measurements of these distances are lacking.

The actual dispersion in dimensions of the nephrons proper, however, is much less than one might expect. In man [*10*] the proximal segments range in length from 12 to 24 mm., the thin limb from 0 to 14 mm., the broad or ascending portion of the distal segment from 6 to 18 mm., and the distal convoluted tubules from 2 to 6 mm. The total length of nephrons run from 20 to 44 mm. In studies of a large number of other mammalian species, Sperber [*11*] found that the proximal tubule makes up from 40 to 60 per cent of the total length in all nephrons and forms the bulk of the renal mass. Glomeruli vary in size to approximately the same extent as the proximal tubules [*9,11*]. Some difficulty in assessing glomerular linear dimensions and mass, results from the tendency for shrinkage and distortion of the capsule to occur during the process of isolation. For practical purposes it may be considered a sphere and volume computed from the diameter. Actually, however, it is more often a flattened ovoid body. The diameters appear to vary within a twofold range, averaging about 37 microns in the mouse, 100 microns in man, and up to 170 microns in the elephant [*9,11–13*]. The range in filtration is probably much greater than this variation suggests because the filtering surface should be a function of capillary length which is related to the volume rather than to the diameter of the glomerulus; i.e., it should vary as the third power of the diameter. A twofold range in diameter, therefore, implies an eightfold range in the area available for filtration. On the basis of the data available [*11,12*], computed glomerular volume appears to be linearly related to the weight of the kidney, suggesting a good correlation between the area of filtration and the mass of the proximal tubule. Thus, in the mouse with 12,430 glomeruli in a kidney weighing 123 mg., the total glomerular volume-kidney weight ratio comes to approximately 3×10^6 μ^3 per mg. whereas, at the other extreme, in the elephant with 7,500,000 glomeruli in a kidney weighing 3,650,000 mg., the ratio amounts to 5×10^6. Whether or not a similarly close correlation obtains within the nephron population of a single kidney has not been established, although unpublished measurements made by Oliver [*17*] indicate that it does.

With disease, the variety of nephron structure becomes more markedly variable. Oliver [*18*] has found that the extremes broaden to include marked hypertrophy on the one hand and atrophic shrinkage on the other, with all gradations between. Depending upon the etiology, focus of injury and rate of progression, glomeruli and the various portions of the tubules are affected variously so that tubular structure is seriously disorganized. In addition, connective tissue replacement of damaged tissue and contraction of fibrous scar tissue result in further disturbances by displacement, obstruction or distortion of glomeruli and tubules, independently of specific etiology. This activity is erratic and focal, although widely disseminated, and its effect is undoubtedly productive of even greater variation in nephron structure and function. The same process acts upon the vasculature and, by interference with the uniform distribution of the blood supply, results in an irregular diminution of filtration. Glomeruli range widely from a wasted vestige, consisting of a few scarcely patent capillaries, to wildly hypertrophic tufts ten times the normal size. Some tubules, seemingly functionless, are aglomerular; others must cope with quantities of filtrate far beyond their capacity. The glomerulotubular balance of functions implicit in the structural correlations in the normal kidney must be completely deranged under these circumstances, although it is possible that the hemodynamic adjustments that control filtration pressure play a compensatory role [*19,20*]. In any case, the obvious exaggera-

tion of anatomical diversity must indicate a corresponding spread in function.

Hemodynamic Causes of Functional Diversity. The formation of urine is essentially a circulatory function, subject to hemodynamic control and to the balance of opposing oncotic and hydrostatic pressures operative across capillary walls. As such a circulatory product, the volume of filtrate may vary widely in association with changes in arterial pressure and intrarenal arteriolar resistances, independently of the area of the capillary surface which is available for fluid and solute transfer. The position of the glomerular capillaries close to the arterial system and between two active arterioles assures at once the maintenance of a high intraglomerular capillary pressure (more than 50 per cent of the peripheral arterial pressure [*7,21*]) and precise local control of flow. No evidence of arteriovenous shunts or other glomerular bypasses have been obtained except in the severely diseased kidney [*18*]. Hence, all the blood perfusing the kidney must first pass through the glomerular capillary bed, and is thus subject to the same controlling influences. However, the peritubular capillary network is freely anastomotic and exclusion of blood from a particular glomerulus does not necessarily interfere with the supply of blood to the remainder of its nephron. The renal vasculature is a remarkably low resistance system into which as much as one quarter of the cardiac output pours each minute. Its organization is such as to assure a fairly uniform distribution of blood throughout the cortex. The blood perfusing the medulla must pass down widely patent straight capillaries into the papillae and return by way of a tight "hair-pin loop" to the interlobular venous outflow [*22*]. The difference in the vascular arrangements in the two parts of the kidney may not only reflect differences in the distribution of blood but also in the character of the extracellular fluid environment. Thus, active regulation of the renal circulation may be a major determinant of nephron activity, both by control of the volume of filtrate which is available to the tubules and by direct influence upon tubular cellular function.

Richards and Schmidt [*1*] were the first to find by direct observation that a fraction of the glomeruli in the frog's kidney might be intermittently unoperative under seemingly normal resting conditions. They saw glomeruli through which blood was flowing vigorously suddenly shut down, presumably as a result of arteriolar vasoconstriction. After a variable period of complete quiesence, active perfusion began abruptly. Alternation between full activity and the complete absence of function could have occurred in such a manner that a relatively fixed proportion of the glomeruli remained operative with resultant constancy in the total glomerular filtration rate. With hydration, or during the action of diuretic agents, a larger number of glomeruli appeared to be in operation at any one time, suggesting the possibility that inactive units made up a kind of functional "reserve." Other workers [*23–25*] soon confirmed these observations in the frog. Further support for the belief that glomerular intermittency might be a general phenomenon seemed to come from the failure to find dye, hemoglobin or other easily detectable materials in a substantial fraction of the glomeruli and tubules soon after injection directly into the renal artery of various animals [*26,27*]. However, on further investigation [*28*], it became apparent that lamination of flow results in an irregular distribution of any particulate material which is introduced directly into the large renal arteries, depending upon the variable factors of branching, eddies and skimming, so that the material may fail to enter some fully functioning glomeruli. In addition, as technics were improved and preparation of the animal was more carefully controlled to avoid undue stress and dehydration, alternation of glomerular activity became less apparent. Under direct examination, mammalian glomeruli were found to be continuously active, although none was watched for more than a very short period [*5*]. However, tubular urine has been sampled on many occasions and found to be formed continuously in all tubules punctured [*5,6,29*]. In the amphibian kidney, also, intermittency now seems to be an unusual manifestation resulting from dehydration, hemorrhage or autonomic stimulation. Even under these conditions, the change in activity is more commonly gradual rather than abrupt, passing from complete glomerular ischemia to a state of sluggish inadequate perfusion of a few capillary loops rather than to full activity [*25,30–32*]. These observations are particularly important because they give substantial support to the view that glomerular filtration is not an "all or nothing" phenomenon. Filtration can be affected not only by changes in the number of glomeruli in operation, but also by change in their activity. A somewhat similar

condition seems to apply to the individual glomeruli, in that filtration depends both upon capillary pressure and surface; i.e., upon the number of capillaries in operation as well as upon the effective filtration pressure.

In the first approximation it seems likely that blood passing from the hilus to the periphery of the kidney must traverse vascular channels having a greater path length, and therefore offering a greater resistance to flow. As a consequence, glomeruli examined directly upon the surface must be precisely those in which perfusion pressure is critically reduced in hypotensive conditions. The resultant state of hemodynamic instability may thus exaggerate the importance of glomerular intermittency. By the same token, however, it follows that arterial hypotension, intrarenal arteriolar constriction, or any other mechanism by which perfusion is reduced, will have a greater effect upon the peripheral than upon the central portions of the kidney. The technic of radiomicrography devised by Barclay [*33*], and used so effectively by Trueta [*22*], Daniel [*34,35*] and their colleagues, yields graphic evidence that this conclusion is valid. Motion pictures of the movement of radioopaque material through the kidney show somewhat more rapid and voluminous perfusion of the medulla than of the cortex, a difference which is greatly increased in experimental animals after blood loss or trauma. This appearance of cortical ischemia is not to be construed as evidence of a shunt or "medullary bypass" that diverts blood from cortical tissue by active vasodilation. It seems rather more likely that intense and widespread vasoconstriction affecting all parts of the kidney simply intensifies the difference in relative perfusion of cortex and medulla. Although suggestive, these data do not prove that the intensity of vasoconstriction varies within the renal arteriolar system. The differences in path lengths may be quite sufficient to account for the contrast between cortical and medullary perfusion. Nor can it be said that similar differences characterize the distribution of blood under "physiologic conditions," but it is clear that distributional changes may indeed be involved in more commonplace adjustments. Non-uniformity in distribution to the various parts of the kidney, to the glomeruli and even to different glomerular capillaries [*36*], coupled with the diversity of nephron structure, would seem to guarantee a considerable variation in glomerular and tubular function, particularly under stress.

The Distribution of Glomerular Activity. To evaluate the effects of irregularities in perfusion, glomerular filtration must be measured in terms of the contributions of the individual units, and blood flow must be assessed in terms of the nephrons, rather than the organ as a whole. The discovery [*7,37–39*] that the tubular transfer of most substances is limited by a maximal rate (transport maximum = Tm) characteristic for each, has provided a means by which this can be done. Glucose Tm and diodrast® Tm have been particularly useful. When the filtered load of glucose (arterial plasma concentration multiplied by the filtration rate) is increased by raising the plasma level, reabsorption increases to the maximum and then becomes constant, the excess glucose filtered appearing in the urine. Diodrast and sodium p-aminohippurate (PAH) behave similarly, but in this case, the load imposed upon the tubules depends upon the arterial plasma level and the renal plasma flow, and when the load rises above Tm, output becomes constant except for the fraction added by filtration. Both reabsorptive and secretory Tm are dependent upon continued filtration, but the difference in loading and transfer mechanisms serves as a useful check. Both transfer systems may be blocked or inhibited by various competing or toxic substances [*7*], but on the whole they have proved to be reliable measures of the functioning mass of nephrons receiving filtrate.

In man [*39*], large changes in urine flow and filtration rate may occur in the absence of significant alteration in either glucose or diodrast Tm. Forster [*40*], in contrast, has found that glucose Tm and urine flow are closely correlated in the frog, possibly indicating an "all or nothing" glomerular intermittency in the intact animal. A number of workers [*41–45*] have recently brought forward evidence that in man, as in the frog, a close correlation obtains between glucose Tm and glomerular filtration rate, both values rising and falling together under a variety of experimental conditions. These reports have been received with some scepticism [*7,46,47*]. Inadequate data have been presented to be certain that the authors have maintained an adequate load, although mention is made in most papers of plasma glucose concentrations that seem to be more than sufficient to assure saturation of the glucose transport sys-

tems. Both reabsorptive and secretory transport maximums have been found to change together in relation to filtration, but inadequate data are presented to indicate that both values fell to the same extent as one would expect if a random sample of the nephron population were removed from function. All these authors [*41–45*] interpret their findings as evidence of "glomerular intermittency." Unfortunately, this expression bears the connotation of an alternation between full and absent activity for which, as noted previously, there is little evidence. The data are all equally compatible with widespread, but irregular, vasoconstriction that has produced an increase in the dispersion of glomerular activity. The failure to achieve maximal transport at the usual plasma levels of glucose certainly implies the development of a more marked diversity in nephron function, some units perhaps being so poorly supplied with filtrate as to be for practical purposes no longer operative. Examination of this possibility requires a more detailed study of the inter-relationship between load and the rate of reabsorption which, as with the simultaneous measurement of secretory Tm, is not yet available.

Theoretically, all the glucose filtered should be reabsorbed during its passage down the tubule until the Tm level is reached, when sugar should begin to appear in the urine, and reabsorption should become constant. In fact, however, as the plasma glucose level is gradually increased ("glucose titration"), glucose begins to appear in the urine at values somewhat less than Tm and reabsorption does not become constant until the load is in excess of Tm. In the dog the glucose threshold is very close to Tm, and plotting reabsorption against load results in close agreement between the observed and theoretical values with very little departure ("splay") at the point of inflection at which reabsorption becomes constant and abruptly stops increasing in direct proportion to load along a straight line with a slope of 45 degrees. Splay is somewhat more marked in man, glycosuria beginning, as a rule, when the load is 60 per cent of Tm and reabsorption leveling off when the load is 50 per cent above Tm. The remarkably close agreement between load and reabsorptive capacity must mean that all the tubules begin to spill glucose at almost precisely the same plasma level and that the variety in nephron population, which is evident anatomically, is minimized functionally by a balance between glomerular and tubular function. Apparently, glomeruli with a high filtration rate are attached to tubules with a correspondingly large reabsorptive capacity, and less active glomeruli to equally inactive tubules, suggesting a structural correlation between glomerular volume and tubular mass in each nephron, like that which seems to hold for the kidney as a whole, from the mouse to the elephant.

The extent of splay and change in threshold is a measure of imbalance or change in the distribution of glomerular activity [*39,48*]. With vasoconstriction that reduces filtration uniformly, much higher plasma glucose levels are required to achieve Tm, but the relationship between load and reabsorption should not be affected. With unequal reduction in filtration that affects some units more than others, the relationship is distorted because higher plasma concentrations are required to saturate glucose reabsorptive mechanisms in those nephrons in which filtration is disproportionately affected. As a result, the splay increases and reabsorption continues to rise as the total load is raised above values usually sufficient to assure saturation. Since the slope at any point on the splayed curve is equivalent to the fraction of the total filtrate that is formed in the remaining unsaturated nephrons [i.e., the derived change in reabsorption (ΔT) divided by the change in load or in plasma level (ΔP) times the total filtration rate (GFR) or ($\Delta T/\Delta P \times GFR$)], the dispersion of glomerular activity relative to tubular capacity may be evaluated for the nephron population in roughly quantitative terms, because it is possible to compute filtration and glucose Tm in each successively saturated moiety. The possibility that the curvature is attributable to an intrinsic change in the character of transfer seems unlikely on the basis of Burgen's [*49*] analysis of the data on the assumption of Michaelis-Menten enzyme kinetics. Changes in the frequency distribution of glomerular activity derived from studies of glucose titration should, therefore, provide reliable information regarding functional diversity among the nephrons arising from structural and hemodynamic irregularities.

Since most disorders of the kidney interfere with glomerular filtration, either by a damaging process as in glomerulonephritis or by circulatory changes as in hypertensive disease, it is not surprising that glycosuria is an uncommon

manifestation of tubular damage. In chronic diffuse glomerulonephritis with far advanced renal damage, the disturbance in glomerulotubular balance may be so profound that a reliable measure of glucose Tm is difficult or impossible [*7, 19, 50*]. It has been found that glucose reabsorption continues to increase as plasma levels of glucose rise far above values which are usually sufficient to assure saturation. This phenomenon indicates a greater reduction in glomerular activity than in tubular capacity in a large proportion of the nephrons, although the population ranges more widely to the opposite extreme in also containing an appreciable number of nephrons that spill glucose at a relatively low plasma glucose concentration. It is also possible that the procedure of glucose titration itself tends to exaggerate the number of inactive units contributing to urine formation by producing an osmotic diuresis which results in detectable excretion by badly damaged nephrons that ordinarily produce little or no urine. A similar response has been encountered among patients in shock in whom urine flow and glomerular filtration are closely correlated when filtration is measured with an osmotically active material like mannitol but not when inulin is used [*51*]. In the course of severe hypertensive disease a similar disturbance in the pattern of glomerular activity may develop, although it is usually not as severe as that observed in chronic diffuse glomerulonephritis [*39*]. Here again, tubular damage is rarely so severe, relative to filtration, that sugar appears in the urine. In contrast, renal diabetes is characterized by glycosuria that seems to be attributable in most instances to an isolated tubular lesion that has never been characterized anatomically. In some instances, a functional imbalance between what appear to be normal glomeruli and tubules, also seems to be at fault [*19,52,53*]. It is possible, of course, that two entirely different disorders are involved, one characterized by a congenital disorganization of nephron structure and one by a discrete lesion of the transfer system. The data are too few, however, to permit appraisal of these possibilities. Nevertheless, the difficulty which has arisen also in the course of studies of the glycosuria and aminoaciduria in Fanconi's syndrome [*54,55*] emphasizes the duality of glomerulotubular imbalance, the necessary involvement of both glomeruli and tubules. In the face of diminished tubular transfer capacity for any substance, perfectly normal glomerular activity becomes excessive and balance can be reattained only by the development of a coordinative reduction in filtration. To some extent, parallel glomerular injury with contraction in the area of the filtering surface suffices, but in addition, "compensation" may require an active reduction in filtration that may, in turn, initiate a vicious spiral of progressive parenchymal damage. Renal disease thus augments structural diversity of the nephrons, and in doing so induces further dispersion in the variation in individual glomerular filtration rates. Furthermore, the "corrective" readjustments are of necessity hemodynamic and almost certain to result in a redistribution of blood flow within the kidney.

The Pattern of Perfusion. An abundance of evidence testifies to a remarkably uniform distribution of blood to parenchymal tissues in the normal kidney. Diodrast and PAH are almost completely absent from the renal venous blood, over a wide range of arterial concentrations in man, and to a large extent also in other animals, when due allowance is made for the amount in the red cells [7,56]. Such an efficient extraction indicates that all the blood must bathe tubular cells which have a high affinity for PAH and diodrast. This does not necessarily imply an even distribution, however, for extraction continues as long as blood perfuses active tissue, regardless of flow in the whole or in different parts of the kidney. Thus, extraction is maintained within normal limits in the face of renal hyperemia during the pyrogenic reaction [*57*] or of ischemia during the action of epinephrine [*58*], and following hemorrhage [*59*], conditions in which there is excellent reason [*22*] to expect redistribution. Similarly, the constancy of diodrast or PAH Tm observed over a wide range of renal blood flows under analogous conditions [7,39] is evidence that *all* the extracting tissue receives an adequate blood supply, but not that all is perfused in a uniform manner. More reliable evidence of uniformity, at least in the resting normal subject, has emerged from studies of diodrast or PAH titration [*7,39*]. As with glucose, the relationship between the load and transfer, determined by gradually raising the blood concentration of diodrast or PAH levels well above Tm, yields information regarding the way in which the test substance is delivered to the cellular transport system. The agreement between theory and observation is even closer than it is with glucose, indicating delivery of

almost exactly the same quantity of diodrast at each level to every extracting cell in the kidney so that the transfer system in each is saturated at approximately the same plasma concentration. In this instance, the splay (which is much less than it is in glucose titrations) is attributable to greater or less perfusion, or loading, of some cells than of others. The slope of the tangent to the curve may be taken as the blood flow to as yet unsaturated tissue at that point and may be used in the construction of frequency distribution curves of relative perfusion rates. However, accurate plotting of the "splayed" curve is difficult and of uncertain validity. In view of the difficulties entailed in the lengthy procedure necessary for adequate titration, the effect of fatigue, repeated arterial punctures, relatively large blood samples, and prolonged immobilization in recumbency with a catheter in the bladder, the almost total absence of splay is very convincing evidence of uniform perfusion of the renal parenchyma under most circumstances. It may be surmised, however, that pyrogenic hyperemia and the intense vasoconstriction of shock will induce a demonstrable increase in splay.

Perfusional redistribution may be accounted for most simply by irregular constriction of the glomerular arterioles so that blood flow is more greatly reduced in some parts of the kidney than in others, or by uniform vasoconstriction that has a greater effect in the periphery owing to greater length of the resisting circuit. A number of other mechanisms have been suggested, but none has been established as definitely operative. Of continued interest, has been the possibility of arteriovenous shunts of some kind by which blood might be diverted from renal tissue. Trueta and his colleagues [*22*] were at first attracted to this notion to explain preferential medullary perfusion under various stressing procedures, but they have since found reason to rule it out [*34,35*]. Certainly the data indicating a high PAH extraction and constant diodrast Tm are opposed to the operation of a shunt in man. In the dog the situation is more uncertain because the PAH that enters the red cell may find its way into the renal venous plasma and may produce falsely low values for extraction. A number of workers [*7,59,60*] agree in finding relatively low values (75 per cent) for PAH extraction in the anesthetized dog, and further studies are necessary to characterize transport more precisely. Recently Pappenheimer and Kinter [*61–63*] have suggested that the redistribution of red blood cells and plasma within the kidney may play a major role in determining renal hemodynamics. In their view, the movement of blood under pressure results in lamination and plasma skimming by vessels branching at right angles. Hence, the blood reaching the periphery of the organ is more cell-rich and viscid than blood perfusing the medulla. As a consequence of the tendency for resistance to increase rapidly as the hematocrit rises above 40 per cent and to decrease but slightly with anemia, the total renal resistance to flow should vary with the arterial pressure, accounting by a simple physical device for autoregulation or maintenance of renal blood flow at a relatively constant rate. Theoretically, anemia should interfere with this mechanism and as a result, filtration should increase in the outermost glomeruli, usually perfused by cell-rich blood, and decrease in the innermost units usually performed with plasma. If this is so, the glucose titration curve should splay markedly as the outermost tubules are saturated at plasma glucose concentrations much lower than normal and the innermost glomeruli at higher levels. However, Kessler, Heidenreich and Pitts [*64*] failed to find that anemia produces any alteration in glucose titration in the dog. Other members of the same group [*60*] were also unable to confirm Kinter and Pappenheimer's [*63*] claim that anemia abolishes autoregulation in the cat. These results are contrary not only to the "cell separation theory" but also to the view that intrarenal vasoconstriction secondary to anemia results in non-uniform distribution of blood to tubular tissue.

With disease, the pattern of perfusion is obviously distorted, although the data are insufficient as yet to permit precise quantitative characterization. The extraction of PAH is usually decreased throughout the course of glomerulonephritis, presumably as a result of perfusion of non-functioning tissue [*19*]. In acute diffuse glomerulonephritis, the combination of renal hyperemia and diffuse impairment of tubular function seems a more likely explanation than either shunting of blood or complete absence of function in large focal areas of the renal parenchyma. As the disease progresses, however, the distortion of the renal vasculature may result in shunting and in restricted anastomotic capillary intercommunication. It may be concluded from these observations [*19*] that the

disease process interferes with circulation, but in nephrosclerosis the reverse seems to be true. In fifteen patients with well established hypertensive disease in whom the renal blood flow ranged from 1,335 down to 633 ml. per minute, PAH extraction remained well within normal limits, presumably as a result of the exclusive perfusion of extracting tissue. This phenomenon suggests that change in tissue function may follow vasoconstriction and redistribution of blood flow [*19*]. The marked depression in PAH extraction observed in malignant nephrosclerosis is consistent with primary tubular damage rather than with an intensification of the vasoconstrictive process that seems to affect the kidney in benign hypertensive disease. Intrarenal vasoconstriction readily occurs even in the presence of far advanced renal disease. Indeed, vasoconstrictive reduction in glomerular filtration may play a more important part in determining functional responses than in the normal, since sodium loss in the face of deprivation is apparently prevented by such a response [*20*]. Vasoconstriction also develops rapidly following assumption of the upright position [*65*]. The fall in glucose Tm under these conditions and the lack of change in the pattern of urinary protein excretion seem to indicate a vasoconstrictive process that effectively eliminates from function a random sample of the nephron population. The transfer maximums, both for reabsorptive and excretory processes, decrease with parenchymal damage. Glucose Tm appears to be more seriously affected in glomerulonephritis than either diodrast or PAH Tm [*7,19,50*]. The marked splay in glucose titration, which is presumably indicative of an extensive and variable depression in glomerular activity, may account in large part for this by interfering with the imposition of an adequate load for determination. The renal blood flow seems to be increased relative to the functioning tubular mass, measured as diodrast or PAH Tm, and it seems likely that tubular tissue is adequately perfused although splay has been reported in the diodrast titration [*50*]. In the nephrosclerotic kidney adequate and uniform perfusion of tubular tissue seems more likely in view of the normal diodrast titration curves [*39*]. This finding, together with the evidence of increased dispersion of glomerular activity and normal PAH extraction, may be interpreted as indicating a diffuse renal vascular lesion in the hypertensive kidney that destroys tissue primarily by the interference with glomerular function. However, the maximal tubular capacity to secrete diodrast is impaired in nephrosclerosis to a considerably greater extent than glucose Tm, in sharp distinction to glomerulonephritis. The lesion is evidently not entirely attributable to the circulatory derangement. Nevertheless, changes in the magnitude and distribution of flows and volumes of blood within the kidney are certainly involved, both in producing and in perpetuating the ill effects of renal injury. The intensified diversity in perfusion and filtration must also exaggerate the functional effects of structural variety among the nephrons.

Intrarenal Delay. In the passage from the glomerulus to the renal pelvis, substances entering the tubules by filtration are delayed a variable period of time, which must depend upon the length and configuration of the nephron as well as upon the manner in which filtrate is processed by the tubule. For a population of nephrons, one may imagine a "population of delay times" each characteristic of an individual nephron, all identical in a completely uniform population or ranging as widely as the individual variation permits. If the population of delay times were uniform, the pattern of change in the plasma concentration of inulin or other similar "glomerular substances" would be faithfully reflected at constant urine flow in the urinary concentrations and the urinary pattern should be superimposable upon the plasma pattern by a simple adjustment in the time axis. The fact that the patterns actually differ greatly may be taken as evidence of the heterogeneity of delay and by extension of function in the nephron population, indeed, it is possible that analysis of differences between urine and plasma concentration patterns could provide a means by which the variation in the nephron population might be characterized in terms of the frequency distribution of "delay times." Two different, but basically similar, approaches have been made upon this problem.

One procedure [*66–69*] used exclusively in human subjects to date, involves analysis of the sequential contributions of filtrate. The test material (inulin is used as the standard) is injected intravenously as quickly as possible and continuous collection of both bladder urine and arterial blood is started immediately. Samples of each are set aside at two minute intervals so that the average arterial concentrations and

urinary excretory rates may be determined simultaneously during ten to twelve successive two-minute intervals. The amount of inulin filtered in each period is computed by multiplying the mean arterial plasma concentration by the over-all inulin clearance ($P_I \times C_{in}$) which is determined later at a constant inulin level maintained by infusion. Then dividing the amount of inulin excreted in the urine during the first period by the amount filtered in that time yields a value for the percentage ($S_1\%$) of filtered inulin which has passed from the glomeruli to the bladder, a figure presumably also referable to the nephrons having the shortest delay times, two minutes or less. Nephrons which are characterized by longer delay contribute in sequence during subsequent collection periods. In the second period, the first sequence of nephrons contribute the same percentage ($S_1\%$) of inulin filtered in the second period ($P_2 \times C_{in}$) and whatever inulin remains must be accounted for by inulin during the first period which has been delayed during its passage down the tubule between two and four minutes. Dividing the quantity of residual inulin by the amount filtered in the first period ($P_1 \times C_{in}$) yields the percentage of filtrate (or nephrons) ($S_2\%$) having a two to four minute delay. In the third collection period both these fractions contribute corresponding percentages of the inulin filtered in the third and second periods, respectively, and the remainder is computed as the percentage of filtrate ($S_3\%$) which has been delayed four to six minutes. Each successive sample is analyzed in this way until all the inulin filtered in the initial period is accounted for and the values of S% are then plotted as a frequency distribution. The shorter the collection periods the more closely one approaches an ideal integration of curves. With ten minute collection periods, the early computations indicated that approximately 75 per cent of the filtered inulin entered the bladder in less than ten minutes, 20 per cent in less than twenty minutes, and the remainder in less than thirty minutes, whereas two minute collection periods yield equivalent fractions for six, eight, and ten minutes, respectively. From the standpoint of equilibration between plasma and urine during the usual clearance procedures, the first figures are accurate in indicating how successive periods of this length are influenced by preceding changes and agree with the work of the Michies [*70,71*] on equilibration. From the standpoint of determining actual delay, the shorter periods yield more accurate results and are a better guide to mean delay which ranges from 4.09 to 6.3 minutes in normal man [*68*]. Still shorter periods might yield still lower values but, in view of an appearance time of two minutes, not really much lower.

More frequent sampling is made possible in dogs by a method devised by Chinard and his associates [*71–74*] which evaluates delay on the basis of the relative renal excretion patterns of various substances. In this procedure the bladder is opened in the anesthetized dog and snugly fitting catheters are inserted well up into the ureters. Collection from each side separately is then made at twelve to thirty second intervals following injection of various substances, singly or in mixtures, directly into the renal artery on one side. Computation consists of dividing the total dose administered into the amount excreted in each period by the "experimental kidney" after subtracting the amount excreted by the "control kidney" to eliminate the effect of recirculation. Plotting these values (weight ratios, w_c) against time yields a frequency distribution curve from which modal (or peak) delay time may be taken and in which nephrons are categorized temporally in terms of the percentage of the dose they excrete. In calculating mean transit time, these values are converted to percentages of the actual quantity of material ultimately recovered from the urine. As with calculation of delay by analysis of sequential contribution, uniform distribution of filtration must be assumed in referring the results to the tubules or nephrons as a whole. In addition, it is necessary to assume that the test material is instantaneously and evenly mixed in the arterial blood so that every portion of the kidney receives it at the same instant and in the same concentration. Moreover, the contribution of any single nephron category to excretion must be limited in duration to one collection period and must not continue thereafter in successive periods. Both methods require the assumption that a steady state is maintained throughout. The mean delay or transit time for the dog ranges from 1.35 to 3.85 minutes for glomerular substances such as inulin or creatinine.

The distribution patterns obtained by these two methods agree closely for inulin, but not for other substances. Following injection of PAH and computation of the fractional contributions sequentially by relating the amount excreted

to the load (i.e., the mean arterial concentration multiplied by the renal plasma flow determined later), the derived distribution curve tallies precisely with that for inulin. This has been interpreted as evidence that PAH is secreted into some portion of the tubule proximal to the point or section which contributes most prominently to total delay. The length of the time intervals employed may interfere with detection of a real difference between PAH and inulin delay. Moreover, calculation of fractional contribution in terms of delivered load requires the assumption of complete extraction from the blood throughout the procedure and does not separate the filtered and secreted fractions in the outflow. Chinard [*74*] has determined the secreted moiety of PAH in the urine following injection into the renal artery as the difference between the simultaneous weight ratios for creatinine and PAH. The differences in weight ratios plotted against time, and taken as equivalent to the distribution of transit times for secreted PAH, yield values for mean and modal transit times in excess of those for creatinine and inulin. Chinard concludes from this that PAH accumulates transiently in the tubular cells during transfer, and he suggests that delay may be occasioned by the relatively slow movement of a macromolecular complex of PAH and intracellular carrier. These computations depend upon the assumptions that the weight ratio for creatinine is equivalent to that for filtered PAH, that analytical error resulting from urine "blank" for inulin or creatinine is negligible and that the delivery of PAH to tubules and glomeruli is equal and uniform.

More serious difficulties arise in evaluating the delay of radioactive electrolytes. In analysis of sequential contributions, the mean arterial and urinary specific activities following injection of Na^{24} or K^{42} are determined and assessed on the assumption that only the difference between specific activities is a function of delay. Thus, $S_1\%$ is calculated by dividing the specific activity of the urine by that of the plasma in the first period, and then assuming that the specific activity of the second urine sample should be the same percentage ($S_1\%$) of the plasma specific activity in that period. The difference between the expected and actual value divided by the specific activity of plasma during the first period yields $S_2\%$, and analysis proceeds in a manner analogous to that employed for inulin. Here it is assumed that specific activity of any radioactive electrolyte in the urine will correspond precisely with that in the blood, unless there is admixture with its non-radioactive isotope. On this basis there is no need to know exactly how much is filtered or reabsorbed. The resorbate will have the same specific activity as the tubular urine and reabsorption will not affect it. Assuming dilution of the radioactive isotope only at the end of the tubule by electrolyte coming from tubules in which delay is longer, the change in specific activity in the urine should be solely a function of plasma specific activity and the pattern of delay. However, it must be assumed, in addition, that a steady state prevails throughout and that the amount of electrolyte coming from each tubule is equal and constant. These assumptions were given support in the initial experiments [*66,67*] by good agreement between distributions of delay for inulin, K^{42}, and Na^{24}. However, it was soon discovered that the presence of PAH resulted in an apparent shortening of the electrolyte delay, presumably as a result of reducing tubular reabsorption of sodium and potassium, with resultant preponderance of radioactive isotope in the urine. In some instances equilibration between plasma and urine specific activity was attained shortly after the first appearance of isotope in the urine. This phenomenon cannot be interpreted as evidence of tubular secretion. Chinard and Enns [*73*] have computed transit times for radioactive water, sodium, and chloride on the basis of weight ratios and have found early excretion relative to creatinine. This they interpret as evidence of a "bypass" of a portion of the tubular system. Delayed excessive output of water and C^{14}-labelled urea, noted in the same studies, was attributed to return to the tubule of a moiety "which has entered tubular cells from the lumen and subsequently returned to the lumen" [*73*]. The extremely small percentage of the total dose recovered in each period raises here, too, the question of the influence of small changes in tubular function. It may be surmised that a high percentage of the dose which does not appear in the urine has been reabsorbed by the tubules after filtration. A decrease in reabsorption in very active nephrons (having a short delay time) would unduly affect the final results. Although both methods have value in defining the variation inherent in the nephron population on the basis of delays which are imposed during the passage of inulin (and creatinine in the dog) down the

tubules, neither appears to be wholly reliable in dealing with other substances.

Appearances can be very deceiving in this connection. Thus, excessive delay, rather than "bypassing," is suggested by the tendency for the urinary specific activity to exceed the arterial specific activity for a prolonged period after intravenous injection of P^{32}, K^{42}, and N^{15}-labelled urea [*66,75–81*]. Govaerts [*75,76*] has suggested that this phenomenon may be explained for phosphate by the presence in the plasma of inorganic phosphate as a non-filtrable and slowly exchanging complex, which falsely reduces plasma specific activity. However, Handler and Cohn [*77*] have found that the plasma inorganic phosphate is freely filtrable and that the discrepancy between urine and plasma can be abolished by keeping the plasma specific activity constant or by making it rise slowly, as expected for intrarenal delay alone. This result does not tell whether delay is longer or shorter than expected for a substance delayed only by the time required to move down the tubules. Black [*78,79*], Morel [*80,81*], and their colleagues believe that the delay in potassium is probably occasioned by movement through the tubular cells into the lumen. As they point out, the tubular cells take up radioactive potassium with great avidity from the blood perfusing the kidney and it might be supposed that subsequent slower release of highly active intracellular potassium would serve to maintain the specific activity of potassium at a higher level in the urine than in the plasma. The apparent difference in the behavior of radioactive sodium (injection of which seems to produce little or no disparity between plasma and urine), and the tendency for the specific activity of potassium in the renal vein (presumably related to cellular activity) to exceed that in the artery, have been put forward as support for this view.

Radioactive sodium and potassium differ in their distribution and in the rapidity with which they enter their respective "pools." As a result, the patterns of arterial specific activities following administration of doses of equal radioactivity are wholly dissimilar. In the first minute or two, both are mixed only in the cardiac output and as a result, during that period the average value of specific activity for potassium exceeds that for sodium by the extent to which the plasma concentrations differ, i.e., by approximately thirty-five fold or 140 to 4 mEq./L. Thereafter, the sodium specific activity quickly stabilizes, because most of the sodium pool is extracellular and readily accessible, the ultimate level approaching approximately one-eighth of the average in the first minute, assuming a cardiac output of 6 L. per minute and a 50 per cent hematocrit. The potassium specific activity reaches equilibrium less rapidly because the isotope must enter the cells, and since the initial value is so much higher than that for sodium, it will fall over a much greater range. These patterns can be assessed only by continuous determination of arterial specific activities. Single determinations, even at frequent intervals, are not sufficient because fleeting but high arterial values may have a striking influence upon the urine which receives, as it were, a continuous sampling of the arterial plasma as glomerular filtrate. For practical purposes, continuous sampling by some device like that employed by Wheeler and his colleagues [*82*] or "integrated" arterial collection is necessary. When this is done and correction is made for the disparities in the two plasma arterial patterns, no significant difference will be evident in the relationships between urinary and plasma specific activities for sodium and potassium [*66*]. The difference in the time of equilibration is therefore more apparent than real and does not support the contention that K^{42} is handled differently by the kidney than Na^{24}.

Accurate delineation of the arterial activity pattern is particularly important in the case of potassium. Approximately 5 per cent of the plasma water and solute passing through the heart each minute passes immediately across the glomerular membrane into the tubules, or approximately 5 per cent of the total dose of radioactive isotope must enter the tubular urine (and almost at once enter the proximal tubule cells by reabsorption) during the first minute after administration. A little less will be introduced in the second minute and much less thereafter as the level falls. The rapid reabsorption of sodium and potassium will result in a speedy rise in proximal cellular specific activity, much more so for potassium than for sodium, because its specific activity is so much higher in the resorbate. In addition, Black, Davies, Emery and Wade [*79*] have found that the specific activity of renal venous potassium equilibrates with that in the artery in about ninety seconds and never reaches high levels, indicating that potassium in the kidney is freely accessible so that K^{42} is quickly distributed in the total renal

potassium content. Indeed, renal venous excess thereafter implies an unequal distribution that leads to "washout" from regions in which an initial high K^{42} content has been established. This phenomenon may be ascribed with equal cogency to washout from the reabsorptive cells which are exposed to initial high levels or to lag in the movement of labelled potassium through the glomerulus, down the tubule, and across the cell into the blood, as to washout from cells secreting potassium. These data are not opposed to the thesis of potassium secretion but it must be admitted that they do not provide conclusive support for it.

Delay affecting the determination of various renal clearances is frequently referred to a "renal dead space." This is a useful simplification, but hazardous, in bearing the implication that some actual ascertainable "space" is involved. In reality, delay is a most complex affair and cannot be ascribed to any structural volume. The term applies simply and strictly only to the substance for which it is determined. Thus, a mean delay time for inulin refers to the average time required for filtered inulin to reach the bladder. The quantity of filtrate initially containing the inulin is much larger than the volume of urine in which it is excreted. Between these two points, the inulin is contained within an ever changing volume of carrier. The mean circulation time through a vascular system, multiplied by the flow per minute, yields a value for the vascular volume because there is no change in volume of carrier or mass of tracer by which the circulation time is measured. This is not true for the renal tubular system. The appearance time (at one end of the frequency distribution curve of nephron delay times), the modal transit time (or the peak) and the mean time do not vary appreciably over a wide range of urine flows [*72,83–85*]. It does not follow from this, however, as some [*72,83–85*] have suggested, that the "dead space" (or urine flow multiplied by time) varies with flow because there is tubular dilatation. In point of fact, the tubules have been observed [*3,5,29*] to dilate during an osmotic diuresis, although no data are available on the effect of water diuresis, the usual method employed in raising urine flow. Nonetheless, the relative constancy of delay times may be said, with somewhat more validity, to imply that the volume of urine flow is determined at some place in the kidney distal to the point of greatest delay. Bojeson [*86*] has avoided this fallacy in his measurement of the volume of the outflow tract by taking the appearance time as a measure of constant intrarenal delay. The good agreement he finds between observed and calculated volumes of the renal pelves may be construed as additional evidence of the relative independence of intrarenal delay and urine flow. Indeed, with the diminished water reabsorption and nephron diuresis of chronic renal disease, the mean delay time measured by analysis of sequential filtrate contributions appears to increase and to splay more broadly to include a greater proportion of prolonged transits [*69*]. The basis for this finding is obscure, although it may be related to a predominance in the nephron population of long, dilated nephrons with reduced glomerular activity. Whatever the ultimate structural and functional causes may be that are responsible for delay, the distribution observed in healthy and diseased kidneys is consistent with the view that nephron activity varies over at least a fourfold range.

Heterogeneity of Nephron Function. An equivalent variety in the urines formed by individual nephrons and pooled in the collecting ducts or renal pelves is suggested, but not conclusively proved, by several data. First, as noted herein, the apparent differences in the delays imposed upon sodium, water, and creatinine may result from differences in the composition of the urines put out by different categories. Excretion of a large volume of urine rich in sodium by nephrons having a short creatinine or inulin transit time would tend to shorten delay for sodium as a whole and by increasing the concentration in the initial periods would improve the chance for early detection and appearance. Disproportionate contributions of short and long nephrons to urine volume might result in the findings reported by Chinard and Enns [*73*] when deuterium- or tritium-labelled water was administered. Secondly, there is some evidence that an excessive carbon dioxide content in alkaline urine relative to the blood may be traceable to admixture of nephron urines of different acidities. Kennedy and his associates [*89*] found that elevation of carbon dioxide tension in mixtures of solutions differing in pH is dependent upon the amount of buffer present (exclusive of bicarbonate) and since urinary carbon dioxide tension and buffer concentration proved to be closely correlated, they concluded that mixing must occur. However, it is also possible that delayed dehydration of carbonic acid formed by

the exchange of sodium for hydrogen ion within the tubule may result in a belated release of carbon dioxide in some portion of the drainage system in which impermeability prevents back-diffusion and equilibration of carbon dioxide tensions between urine and blood [*87*]. Since infusion of carbonic anhydrase will abolish the disparity [*90*], delayed dehydration (attributable to insufficient enzyme, to "competition" of the buffer for hydrogen ion [*91*] or to both) is probably also implicated or admixture occurs at some point where surface-volume relationships are transiently favorable to back-diffusion. These tantalizing hints that heterogeneity of function may characterize the nephron population, raise the question whether or not change in urinary composition may not occasionally reflect alteration in the contributing populations, rather than in intrinsic tubular or glomerular function. Such a possibility certainly cannot be dismissed whenever hypofiltration is associated with diminished transfer maximums.

In fact, elevation of intra-abdominal pressure in man induces changes in urine formation that could be accounted for in part by this mechanism [*92–94*]. Abdominal compression by a pressurized girdle has been found to produce a uniform increase in pressure throughout the abdomen and its hollow viscera. At 20 mm. Hg urine flow and electrolyte excretion decrease markedly in company with a diminution in renal blood flow and filtration rate. Vasoconstriction does not occur and the reduction in blood flow can be attributed to an elevation in venous pressure [*92,95*]. Venous congestion probably does not occur to any extent owing to the general reduction in transmural pressure, although it is possible that venous collapse in some regions may conduce to irregularities in the distribution of the venous blood volume [*96*]. Reduction in renal blood flow without change in arteriolar resistance should result in a simultaneous rise in intraluminal glomerular capillary pressure that would counter the rise in renal interstitial tension and maintain filtration at, or even above, the central value. The fall in filtration is associated with, and explained, by a proportionate and simultaneous decrement in both glucose and diodrast Tm that must denote cessation of function in a corresponding fraction of the nephrons. In this interpretation of the findings, the function in the residual active nephrons should be unaffected. Although the kidney is theoretically non-compressible, in the presence of a drainage system to carry away urine by active peristalsis, a tubular transport system that can reduce intraluminal content, and venous outflow by which blood volume may be transferred, a considerable degree of compressibility is in fact possible. It is conceivable that compression of the papillae and elevation of the renal pelvic pressure may block outflow from some collecting ducts and not from others [*97*]. In such a case, the effect would probably not be random, but would involve those units with the least turgor or residual energy content. If this interpretation is correct, the resultant inoperative nephrons are randomly distributed through the kidney, since PAH extraction is not affected, although alike from a functional standpoint.

On compression, the water and sodium output decrease, sodium more than water, so that the urinary sodium concentration falls. A rise in the inulin, or mannitol, urine-plasma ratio indicates greater reduction in urine flow than in filtration. Change in the potassium output, as expected [*98*], parallels that in filtration. Since this response has been observed in patients with diabetes insipidus [*93*] and after total adrenalectomy [*99*], it cannot be attributed to release of ADH or of adrenal cortical hormones. It is interesting to speculate on the possibility that the fraction of nephrons no longer in operation had been elaborating a relatively large volume of sodium-rich urine prior to compression. Withdrawal of their contribution from the outflow would produce the modifications or urinary volume and composition that were observed without requiring any change in tubular function. It seems unlikely, however, that cessation of nephron function would be an "all or nothing" phenomenon and that the residual active units are not affected by the block to outflow. A similar effect is produced by an elevation in renal venous pressure alone [*100*], possibly because intrarenal venous engorgement acts similarly to interfere with urinary drainage. With compression, venous distention seems improbable, but the similarity of the responses does suggests a similar underlying mechanism of obstruction, operating in part perhaps to change the time of contact between filtrate and tubule cells and in part by removing a nonrandom fraction of nephrons from function.

Recently, Malvin, Sullivan and Wilde [*101*] have suggested that temporary obstruction to urinary outflow may permit the establishment

of concentration maximums along the tubules that persist when occlusion is released and that may be used, when properly plotted with regard to outflow and time, as a guide to localization of function in the tubules by "stop-flow analysis." The authors assume that filtration ceases immediately after obstruction to outflow from a catheter placed in the ureter and they plot their data in a sequence of equal intervals assigning equal weight to each collection of the same volume. It is difficult to believe, in light of the data reviewed here, that urine flow ceases abruptly on stopping ureteral outflow and that accumulation does not occur in the pelvis of the kidney. The convention of plotting concentrations in successive equal volumes of urine has the effect of unduly exaggerating the first few seconds of collection, after release, when there must be an outrush of urine penned in the pelvis. Thus, on the basis of Bojeson's [*86*] values for pelvic volume, the first ten to twelve points are, in all likelihood, referable to urine in the pelvis actually excreted during the period after occlusion, before pressure had increased sufficiently to halt outflow. As expected from the data on abdominal compression, the sodium concentration decreases and the creatinine urine-plasma concentration ratio rises. The other changes observed may be referable to successive contributions of different categories of the nephron population and it is conceivable that this method may provide an approach to the analysis of the functional heterogeneity in the nephron population.

This survey of information available on the structure and function of the nephron seems to belie the common assumption of uniformity which is implied in the equation of functions of the kidney as a whole with function of a single unit. Anatomically the nephrons are notably multifarious, in dimensions, mass, configuration, construction and arrangement. The glomerular intermittency with alternation of function between complete inactivity and full operation which has been observed directly does not seem to be a cause for variability under normal conditions. However, circulatory adjustments may involve at least a partial, and not altogether uniform, reduction in filtration and perfusion that should enhance whatever tendency to functional heterogeneity the variety in structure connotes. Disease and injury contribute in this way, too, by inducing hemodynamic as well as tissue derangements. The effect of variation in glomeruli and proximal tubules is largely minimized by a balanced and symmetrical organization that is manifest in a close correlation between glomerular activity and tubular reabsorptive capacity during glucose titration. Intersegmental balance may also operate to reduce variation. Nephron function expressed as "nephron delay time" varies normally over a fourfold range, more widely, indeed, than body weight or stature. With disease, delay ranges still more widely. Much more work must be done to define these disparities and modulation. Mass demographic methods should be applied in anatomic studies of the nephrons. The available data are too few and too highly selected to yield reliable populational statistics. Direct measurements of nephron function by micropuncture technic in the number, simultaneity, and accuracy required are at present obviously impossible. Perhaps suppression of activity selectively in a single nephron category would afford one means of "functional dissection" by which diversity could be assessed quantitatively. Efforts to do this have yielded equivocal results so far either because a random sample of nephrons is affected or because contravening adjustments may occur in the residual active units. In any case, the data now at hand speak strongly for a multiplicity of nephron activity that must be taken into account in the evaluation of any change in urine formation.

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Non-ionic Diffusion and the Excretion of Weak Acids and Bases*

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Some weak acids or bases are concentrated on one side of a biological membrane provided there is a hydrogen ion gradient between the two sides. Concentration occurs by purely physicochemical diffusion processes, and is dependent on a greater permeability of the membrane to the unionized than to the ionized fraction. No active transport system and no energy other than that required to maintain the pH difference is involved. Weak bases tend to accumulate in the more acidic fluid, and weak acids in the more alkaline. A familiar example is the accumulation of ammonia in acid urine within the distal tubule. For convenience this type of diffusion process will be referred to in this paper as "non-ionic diffusion," a term which emphasizes the fundamental importance of the greater permeability of the cell membrane to the unionized component of the weak acid or base.

The permeability of a cell membrane to any substance is dependent on the properties of the membrane and on the type of penetrating molecule. The most important molecular characteristics involved are the molecular weight and dimensions, and the nature of individual groups of the molecule. If there are many polar groups, e.g., —OH and —COOH, the substance will tend to be water-soluble, whilst the presence of non-polar groups, e.g., $—CH_3$, makes the substance more likely to be soluble in organic solvents. In the last decade of the nineteenth century Overton [*1*] showed that molecules with a high oil/water partition coefficient were able in general to penetrate cell membranes more easily and rapidly than those with a low partition coefficient. He suggested that the cell membrane is composed of a thin lipoid layer allowing the entry of oil-soluble compounds. Although several refinements have been added [*2*], this theory has been confirmed by later research. The rate of diffusion usually increases with smaller molecular size, greater lipoid solubility, and the presence of non-polar groupings in the molecule. This review is chiefly concerned with the penetration of weak bases and acids in which the unionized fraction possesses these properties and thus is highly diffusible. Penetration of the ionized fraction is impeded by the electrical charge and the water solubility of this component.

There is some hydrogen ion gradient across most cell membranes, the intracellular pH tending to be lower than that of the extracellular phase [*3,4*]. This doubtless influences diffusion and concentration of weak acids or bases in the cell. It is, however, easier to investigate systems where the cells separate two fluid phases of widely different pH. This occurs in the gastric mucosa, pancreatic acini and the renal tubules. It is advantageous to consider all these, since many general principles can be transferred from one system to the others. Weak bases will be found in higher concentration in acid gastric juice than in plasma, acids in less concentration. The reverse applies in alkaline pancreatic juice. Weak acids will tend to be excreted more rapidly in alkaline urine, bases in acid urine. This paper reviews the quantitative aspects involved.

Weak bases and acids include many substances of great physiological and pharmacological importance. Since the ionized and unionized fractions may have widely different effects and activity, their behaviour is often greatly influenced by small changes of pH. An important example is the variation in excretion produced by change of urinary pH. Table 1 shows that only a small number of weak acids and bases are known to be excreted in a manner characteristic of non-ionic diffusion. This is explained by the theoretical aspects of the diffusion process described in the next section. These few substances however provide an important exception to the

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classical principles of renal physiology involving glomerular filtration followed by selective tubular reabsorption or secretion.

Theoretical Considerations. In aqueous solution, weak bases and acids are partly composed of dissociated and partly of undissociated molecules, the proportion of each being a function of the pH of the fluid. This relationship is expressed in the well known Henderson-Hasselbalch equation:

$$pH - pK_a = \log \frac{I}{U} \qquad \text{(acid)}$$

or $$pH - pK_a = \log \frac{U}{I} \qquad \text{(base)}$$

where pK_a is the negative logarithm of the dissociation constant, and U is the concentration of the unionized fraction and I of the ionized fraction. When $pH = pK_a$, $I = U$, and the concentration of each fraction is 50 per cent of the total. For the purpose of calculation of concentration ratios produced by non-ionic diffusion, the corresponding exponential forms of these equations are often more useful:

$$I = U.10^{(pH - pKa)} \qquad \text{(acid)}$$

or $$I = U.10^{(pKa - pH)} \qquad \text{(base)}$$

In general, weak bases are more often involved in processes of non-ionic diffusion than weak acids, because the highest hydrogen ion gradients are those between plasma and acid gastric juice or acid urine. The following derivation of concentration ratios will be confined to weak bases, but similar principles can readily be applied to weak acids. All the figures refer to bases except Figure 2 which applies to weak acids.

Derivation of theoretical concentration ratio at equilibrium between fluids of different pH, assuming free permeabiliy to the unionized fraction and complete impermeability to the ionized fraction: These equations were first derived by Jacobs [*5*], and have recently been applied by Shore et al. [*6*] to concentration ratios of weak bases and acids between gastric juice and plasma. The concentration ratio (R) of a weak base between fluids m and n of different pH (pH_m and pH_n) is obtained as follows:

$$R = \frac{U_n + I_n}{U_m + I_m} = \frac{U_n + U_n.10^{(pKa - pH_n)}}{U_m + U_m.10^{(pKa - pH_m)}}$$

but at equilibrium U_m is assumed to be equal to U_n, and therefore:

$$R = \frac{1 + 10^{(pKa - pH_n)}}{1 + 10^{(pKa - pH_m)}} \qquad (1)$$

Similarly for a weak acid:

$$R = \frac{1 + 10^{(pH_n - pKa)}}{1 + 10^{(pH_m - pKa)}} \qquad (2)$$

These equations (1) and (2) contain four variables, R, pH_m, pH_n and pK_a. However, when diffusion from plasma to gastric juice or urine is considered, pH_m is relatively constant at pH 7.4. Figures 1 and 2 are the graphs of these equations for weak bases and weak acids respectively. R is plotted as ordinate and pK_a as abscissa. The weak base or acid is considered to be diffusing from plasma into urine of pH varying from 4.4 to 8.4, and into gastric juice of pH 1.4. Weak bases are concentrated into the fluids more acid than plasma, and weak acids into those more alkaline than plasma. The concentration ratio rises as the pH difference between the fluid and plasma increases.

If this simple mathematical analysis were directly applicable to the diffusion of bases from plasma into gastric juice, concentration ratios as high as 10^6 would be predicted, whereas 40 is the highest ratio actually found. Similarly in the case of urinary excretion of weak bases of pK_a above 9.4, the clearance would be expected to increase ten-fold for every unit fall of urinary pH. In actual fact the usual rate of increase is only one third this amount. Observed concentration ratios are always lower than those theoretically predicted by the equations cited because three invalid assumptions have been made. These assumptions are: (1) The membrane is completely impermeable to the ionized component. Whilst there is abundant evidence that there is greater permeability to the unionized component than to the ionized, there is no proof of complete impermeability to the latter. (2) Complete equilibrium is reached during the time available for diffusion. Most biological processes have to take place in a limited period of time and thus there is often incomplete equilibration in diffusion processes. (3) The blood flow is infinitely large compared to the flow of gastric juice or tubular fluid. Concentration ratios are measured, in practice, between gastric juice or tubular fluid and arterial plasma.

The true concentration ratio is that between fluid and capillary plasma at the diffusion site. Since the rate of blood flow is not infinitely large compared to the rate of flow of gastric juice or tubular fluid, the concentration of the weak base in capillary plasma will always be lower than in arterial plasma.

The following three sections attempt to quantitate the separate limiting effect of each of these factors on the theoretical maximum concentration ratio shown in Figures 1 and 2. The combined effect of the three factors operating simultaneously is unfortunately too complex for analysis in the present state of knowledge.

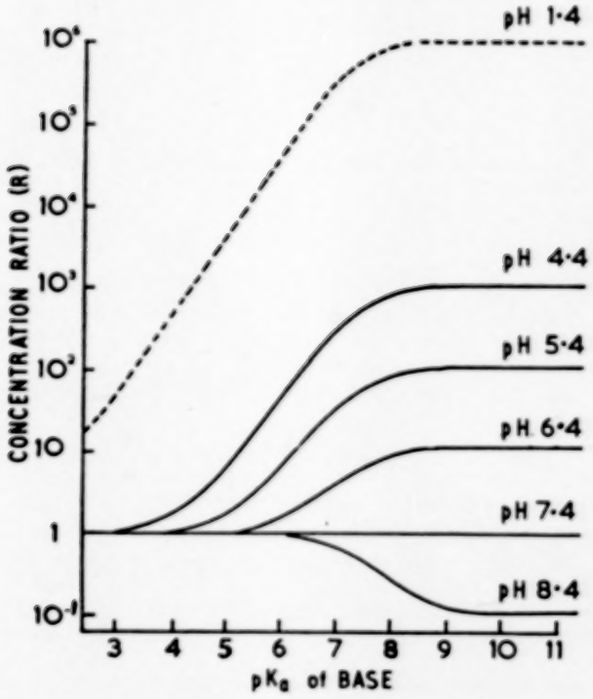


FIG. 1. Theoretical urine/plasma (continuous lines) and gastric juice/plasma (broken line) concentration ratios at equilibrium for bases of various pK_a values. The plasma pH is assumed to be 7.4 and the pH values of urine and gastric juice are as indicated. It is assumed that there is free permeability to the unionized fraction of the base and complete impermeability to the ionized fraction.

1. *Derivation of concentration ratio of a base at equilibrium, assuming free permeability to the unionized fraction and partial permeability to the ionized fraction:* The following equations were derived by Orloff and Berliner [7] in their considerations of the excretion of ammonia and other weak bases. The unidirectional rate of flow of a substance across a membrane of unit surface area is given by the product of the concentration of that substance and the permeability coefficient. Let F_{mn} represent the rate of flow from fluid m to fluid n, and F_{nm} the rate of flow in the reverse direction. K_U is the permeability coefficient of the unionized fraction and K_I of the ionized fraction.

$$F_{mn} = K_I.I_m + K_U.U_m$$

and

$$F_{nm} = K_I.I_n + K_U.U_n$$

At equilibrium there is no net flow across the membrane, and therefore

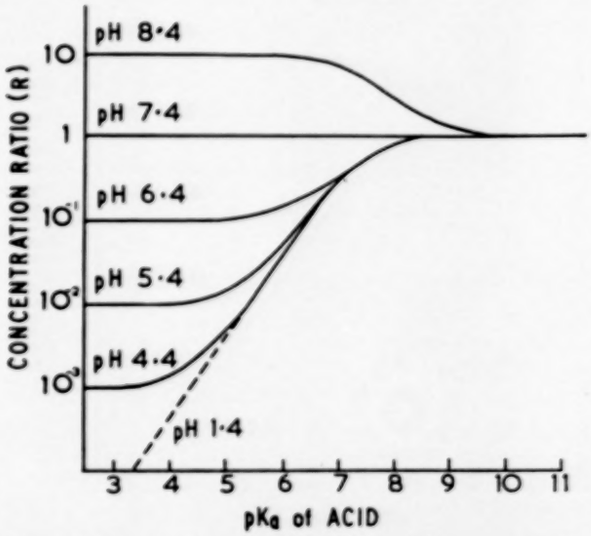


FIG. 2. Theoretical urine/plasma (continuous lines) and gastric juice/plasma (broken line) concentration ratios at equilibrium for acids of various pK_a values. Plasma pH is assumed to be 7.4 and pH values of urine and gastric juice are as indicated. It is assumed that there is free permeability to the unionized fraction of the acid and complete impermeability to the ionized fraction.

$$F_{mn} = F_{nm}$$

Whence $K_I.I_m + K_U.U_m = K_I.I_n + K_U.U_n$

$$\frac{K_U}{K_I} = \frac{I_n - I_m}{U_m - U_n} = \frac{U_n.10^{(pK_a - pH_n)} - U_m.10^{(pK_a - pH_m)}}{U_m - U_n} \quad (3)$$

The fraction K_U/K_I is the ratio of the permeability coefficients of the unionized and ionized fractions respectively. Since both coefficients are positive and $K_U > K_I$, the ratio could have possible values ranging from unity to infinity. If there is no preferential permeability to the unionized fraction, $K_I = K_U$, and consequently $I_n + U_n = I_m + U_m$. Therefore the total con-

centration of the weak base would be the same on both sides of the membrane despite a hydrogen ion gradient. At the other extreme, when the ratio is infinity (complete impermeability to the ionized fraction), $U_m = U_n$, and the relationship derived in the previous section holds good.

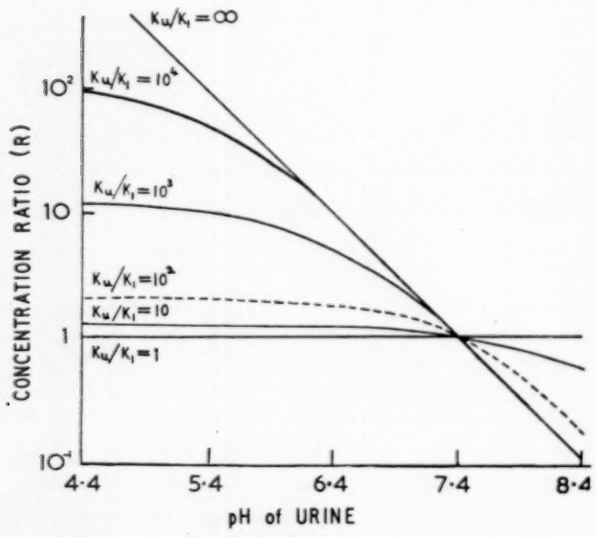


FIG. 3. Theoretical concentration ratio at equilibrium of a base of pK_a 9.4 between urine of varying pH and plasma of pH 7.4. When there is complete impermeability to the ionized fraction ($K_U/K_I = \infty$) log R is inversely proportional to urinary pH, and R increases ten-fold for every unit fall of pH. When the permeabilities of the ionized and unionized fractions are equal (K_U/K_I = 1) the concentration ratio is unity. At intermediate levels of K_U/K_I (K_U/K_I = 10, 10^2, 10^3 and 10^4) there are intermediate values of R, the relationship between log R and pH being curvilinear.

This will result in the theoretical maximal concentration ratio of Figures 1 and 2. At intermediate levels of K_U/K_I, submaximal concentration ratios of the weak base will be obtained. These can easily be calculated at any given value of K_U/K_I from equation (3).

The introduction of the permeability ratio (K_U/K_I) adds another variable, and therefore graphs can be constructed only if one of the four varying factors is kept artificially constant. Thus in Figure 3 it is assumed that the pK_a of the diffusing base is constant at 9.4. This figure shows the limitation of concentration ratio produced by permeability to the ionized fraction. If K_U/K_I is infinite the concentration ratio increases tenfold for every unit fall of urinary pH. Log R is therefore a linear function of urinary pH with a gradient (d log R/d pH) of minus one. If the permeability coefficients are equal (K_U/K_I = I), there is no concentration gradient of the weak base across the membrane and R is unity. At intermediate levels of K_U/K_I there is a curvilinear relation between log R and pH, but this approaches a linear relation at higher urinary pH values. The reduction of the concentration ratio from the theoretical maximum is greater at lower values of K_U/K_I.

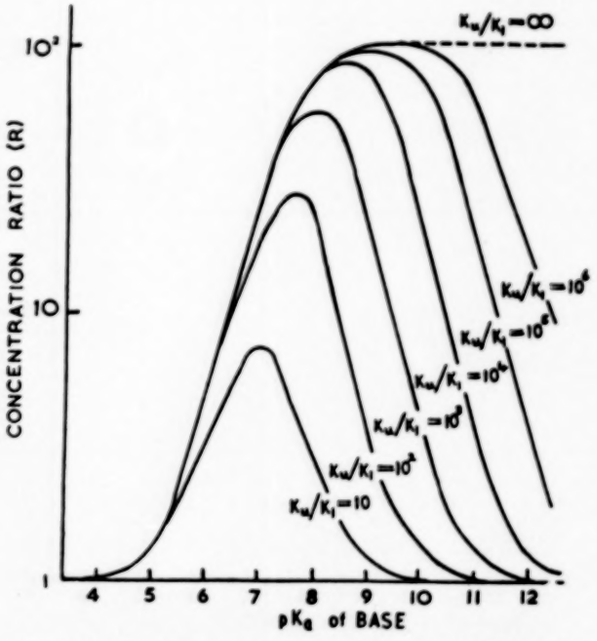


FIG. 4. Theoretical concentration ratio at equilibrium of bases of varying pK_a between urine of pH 5.4 and plasma of pH 7.4 at different values of the ratio of the permeabilities of the ionized and unionized fractions (K_U/K_I = 10, 10^2, 10^3, 10^4, 10^5 and 10^6). The broken line gives values of R if K_U/K_I is infinite and is identical with the line labelled 5.4 in Figure 1. It is seen that adequate concentration into acid urine is obtained with bases of pK_a from 6.5 to 10 at relatively low values of K_U/K_I. With bases of higher pK_a, concentration is only obtained with very high values of K_U/K_I.

In Figure 4, an arbitrary value of pH 5.4 has been assumed as representative of an average acid urine, and the effect of simultaneous variation of the pK_a of the base and of K_U/K_I is examined. The curve for $K_U/K_I = \infty$ is identical with that labelled 5.4 in Figure 1, and again represents the theoretical maximal ratio if there is complete impermeability to the ionized fraction. At lesser finite values of K_U/K_I curves approximating in form to a normal distribution curve are obtained. It is seen that there is an optimum range of pK_a between 6.5 and 10 at which a considerable concentration into acid urine is obtained at relatively low

TABLE I*

Weak Base or Acid	pK_a	Plasma Protein Binding (%)	Amount Excreted Unchanged (%)	Clearance Ratio $\frac{C_x}{C_{in}}$		K_U/K_I	Remarks and References
				Acid	Alkaline		
Ammonia	9.3	.....	.....	Excretion High	Excretion Low	$>10^4$	.....
Quinacrine	7.7	80–90	4–11	3.0	.5	$>10^2$	Total plasma clearance, man [*133*]
Chloroquine	8.05	55	10–20	High	Low	$>10^2$	Exact figures not available
Quinine	8.3	70	5	.65	.05	$>10^3$	Total plasma clearance, dog [*7*]
Procaine	8.95	30	2	2.25	0.25	$>10^4$	Unbound plasma clearance, dog [*82*]
Mecamylamine	11.3	28	60	4.6	0.06	$>10^5$	Total plasma clearance, dog [*63*]
Salicylic acid	3.0	75	10–85	.02	1.6	$>10^5$	Unbound plasma clearance, man [*88*]
Phenobarbital	7.2	40	25	.1	.7	$>10^2$	Unbound plasma clearance, dog [*74*]

* Weak acids and bases excreted by non-ionic diffusion. The ratio of permeabilities of the unionized and ionized fractions (K_U/K_I) are calculated from Figure 4 in the case of bases and from a similar figure for the acids.

values of K_U/K_I. With the one exception of mecamylamine, all weak bases known to be excreted by a diffusion mechanism fall into this range of pK_a. (Table I.) Mecamylamine has a pK_a of 11.3, and Figure 4 shows that the renal tubule cells must be highly impermeable to the ionized fraction of this base. The ratio K_U/K_I must be at least 10^5 for significant concentration to occur. Similar considerations in the case of weak acids diffusing into alkaline urine show that the optimum pK_a range is 5.0 to 7.5. For bases diffusing into acid gastric juice the optimum range is 3.5 to 9.0. Again mecamylamine is an exception and the same high figure of $K_U/K_I = 10^5$ must be assumed to explain the significant concentration which actually occurs. (Table II.)

2. *Reduction of concentration ratio by rate limitation of the diffusion process:* In the language of pure mathematics every quantity is infinitely divisible; consequently the process of diffusion never reaches absolute equilibrium. The chemist however considers equilibrium to be reached for practical purposes when it is no longer possible to measure a difference of concentration in the two phases, and knows that true equilibrium actually occurs when the gradient is approaching molecular dimensions. In the following mathematical analysis of rate limitation of non-ionic diffusion it will be shown that apparently infinitesimal concentration gradients may be of practical importance.

By Fick's law of diffusion, the rate of transfer of any diffusing substance $\left(\frac{dQ}{dt}\right)$ is proportional to the area of the surface membrane (A) and to the concentration gradient $(U_m - U_n)$.

$$\frac{dQ}{dt} = K_U.A.(U_m - U_n),$$

where K_U is the diffusion coefficient of the unionized component.

But $Q' = V(U_n + I_n) = U_nV\{1 + 10^{(pK_a - pH_n)}\}$

where V is the volume of fluid into which the substance is diffusing.

Therefore: $\frac{dU_n}{dt} = \frac{K_U.A(U_m - U_n')}{V\{1 + 10^{(pK_a - pH_n)}\}}$

which on integration gives:

$$\ln \frac{U_m}{U_m - U_n} = \frac{K_U.A\ t.}{V\{1 + 10^{(pK_a - pH_n)}\}}$$

or

$$\log \frac{U_m}{U_m - U_n} = \frac{0.4343\ K_U.A\ t.}{V\{1 - 10^{(pK_a - pH_n)}\}}$$

Therefore if $\log \frac{U_m - U_n}{U_m}$ is plotted against

TABLE II*

Weak Base or Acid	pK_a	Plasma Protein Binding (%)	R (observed)	R (corrected for binding)	Maximal Possible Value of R	K_U/K_I
Bases:						
Acetanilide	0.3	0	1.0	1.0	1.0	.....
Theophylline	0.7	15	1.5	1.3	1.5	.....
Antipyrine	1.4	0	4.2	4.2	4.2	.....
Aniline	5.0	25	40	...	10^4	>10
Aminopyrine	5.0	15	42	...	10^4	>10
Quinine	8.4	75	38	...	10^6	$>10^2$
Levorphan	9.2	50	40	...	10^6	$>10^3$
Tolazoline	10.3	23	10.5	...	10^6	$>10^4$
Mecamylamine	11.3	28	20–40	...	10^6	$>10^5$
Acids:						
Salicylic	3.0	75	0	0	10^{-4}	.....
Probenecid	3.4	75	0	0	10^{-4}	.....
Phenylbutazone	4.4	90	0	0	10^{-3}	.....
Thiopental	7.6	75	0.12	0.5	0.6	.....
Barbital	7.7	0	0.6	0.6	0.6	.....
p-Hydroxypropio phenone	7.8	75	0.13	0.5	0.6	.....

* Weak bases and acids diffusing into gastric juice when given intravenously. The ratios of the permeabilities of the unionized and ionized fractions (K_U/K_I) are calculated from a similar figure to Figure 4. Values except those for mecamylamine [*24*] are taken from the paper of Shore et al. [*6*].

time, a straight line with a slope equal to minus $\frac{0.4343\ K_U.A\ t.}{V\{1 + 10^{(pK_a - pH_n)}\}}$ is obtained. Provided the pK_a of the base is more than two units above pH_n the slope will decrease ten-fold for every unit fall of pH_n. Consequently it will take ten times longer for the same degree of equilibrium to occur. If only a certain fixed time is available for diffusion, there will be a much greater deviation from the equilibrium state at low values of pH_n at which there is a greater transfer of the diffusing base.

The three curves in Figure 5 graphically represent the limiting effect of three finite diffusion rates on the theoretical maximum concentration ratio (straight line) for a base of pK_a above 9.4. The assumption is made that the time available for diffusion is unaffected by change of urinary pH. As urinary pH decreases, the disequilibrium between U_m and U_n rises, causing a corresponding reduction in the concentration ratio of total base. For every unit decrease of pH_n there is a tenfold increase in the amount of base which must diffuse before equilibration is complete. The lowest curve shows the effect of a slow diffusion rate which reaches 90 per cent of full equilibrium at pH 7.4. With equal diffusion time, this same rate limits the equilibrium at pH 4.4 to 0.23 per cent of completion with a correspondingly large reduction of the concentration ratio (R) from 10^3 to 2.3. The highest curve shows the effect of a very rapid rate of diffusion which, at a urinary pH of 7.4, permits the equilibrium between U_m and U_n to come within $\frac{1}{10^{100}}$ of completion. However, at a pH of 4.4 equilibrium between U_m and U_n is still only 23 per cent complete, with reduction of R from 10^3 to 230. At pH 4.4, 1,000 times more base must diffuse across the membrane than at pH 7.4 to produce the same degree of equilibration. It is obvious therefore that any substance showing a significant degree of concentration by non-ionic diffusion must pass across cell membranes with great rapidity.

It is seen from Figure 5 that the curvilinear regression between log R and urinary pH approaches a maximum at lower pH values. This fact epitomizes the difference between diffusion of bases into neutral and highly acid urines. At the start of diffusion the driving force is identical, being the concentration of the unionized base in the plasma. In neutral urine the rate of diffusion rapidly falls off as the concentration of the unionized fraction in the urine rises. In acid urine almost all the unionized molecules diffusing across the tubule cells become ionized when they pass into the acidic tubular fluid. A high

rate of diffusion is therefore maintained throughout the whole of the diffusing period. The maximal possible transfer is that obtained if the rate of passage remains constant at the high initial value during the whole of the available time.

3. *Apparent reduction of diffusion concentration ratios due to false reference to arterial instead of capillary plasma:* In order to measure a concentration ratio of any substance across a membrane it is obviously necessary to determine the separate concentrations of the substance in the two phases. Unfortunately this is not always possible in biological systems and then only an estimate of the true ratio can be made. In the stomach, for example, the true ratio is that between the gastric juice and the non-protein bound fraction of the substance in mucosal capillary plasma after diffusion is complete. The concentration in the juice can be measured directly, usually by obtaining samples from a Heidenhain fundic pouch in a dog under maximal stimulus from a histamine infusion [*6*]. The postdiffusion plasma concentration can only be inferred and is always lower than any plasma concentration that can be directly measured. The level in arterial plasma is obviously higher since diffusion has not taken place. The concentration in gastric venous blood is also higher than the true value since there has been admixture with an unknown proportion of blood which has perfused gastric muscle and connective tissue, and from which no diffusion has occurred. The net effect is a measured concentration ratio which is falsely low, the error increasing with high rates of transfer of the diffusing base.

In the kidney the situation is even more unsatisfactory as neither phase is available for direct analysis. As will be shown later, the site of non-ionic diffusion in the nephron is proximal to that of water reabsorption in the distal tubule. The concentration of the diffusing base in urine in the bladder is therefore higher than the average value in tubular fluid at the diffusion site. The nearest approximation to the true value is the concentration in urine obtained during conditions of maximal water diuresis. Since more is known about the dynamics of the renal than of the gastric circulation, it might be thought that a more accurate estimate could be made of the concentration in the plasma perfusing the renal tubules. Current uncertainties in renal physiology however make such an estimate of doubtful validity. Studies of renal haemodynamics indicate that about 92 per cent of the renal plasma flow actually perfuses the nephrons [*8*] and is therefore available for diffusion. Pappenheimer and Kinter [*9–11*] have presented evidence that a proportion of the erythrocytes

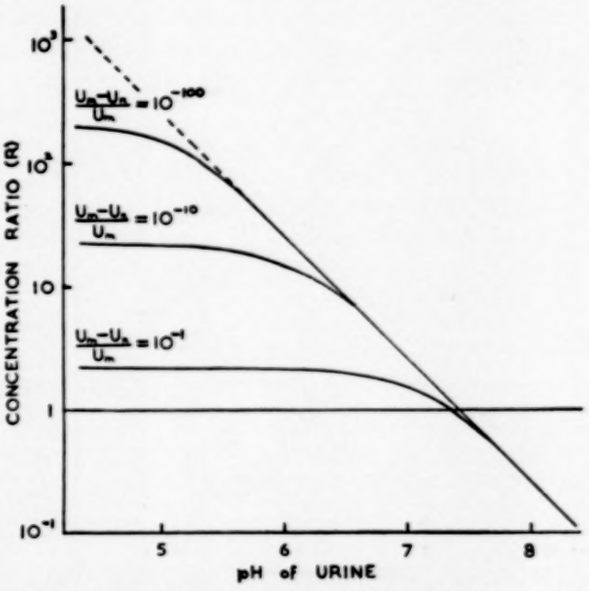


FIG. 5. The effect of rate limitation of diffusion on the concentration ratio of a base of pK_a 9.4 between urine of varying pH and plasma of pH 7.4. The lowest curve shows the gross reduction of R produced by a slow rate of diffusion which reaches 90% of full equilibrium at urine pH 7.4. The highest curve shows the appreciable reduction of R at a rapid diffusion rate reaching within $\frac{1}{10^{100}}$ of complete equilibrium at urine pH 7.4. The straight line shows the theoretical maximum values of R at infinite diffusion rates.

may be separated off by "plasma skimming" and shunted into the renal veins without being in contact with the renal tubules. Bradley's criticisms [*12*] of the data show that this must be regarded as a feasible hypothesis rather than undisputed fact. All substances which are excreted by a diffusion mechanism pass freely into cells and are present in erythrocytes often in a concentration higher than that of plasma. If separation of erythrocytes does occur, the concentration of the diffusing base within renal capillary plasma will be much lower than if the tubules are perfused with whole blood. In either case, however, analysis of renal venous blood will give an identical result since a new state of equilibrium between plasma and erythrocytes is rapidly reached. The exact degree of protein binding within the capillary plasma is a

further unknown factor. Most of the substances concerned are partially protein-bound (Table I), and the proportion bound usually considerably increases as the total concentration falls [*13*].

An approximate relationship between the true concentration ratio (R) and the apparent concentration ratio (R_a) between tubular fluid or gastric juice and arterial plasma can be derived as follows: Let V_m be the rate of blood flow at the diffusion site, and V_n the rate of flow of either gastric juice or intratubular fluid. C_{cap} is the average concentration of weak base in capillary blood, and C_a in arterial blood. The ratio of the concentration of the non-protein bound fraction to the total concentration is b, and here it will be assumed that b is constant. If b varies, similar but rather more complex relationships can be derived. The amount of base delivered by the arterial blood in unit time is $V_m.C_a$. This is distributed between capillary blood and either gastric juice or tubular fluid.

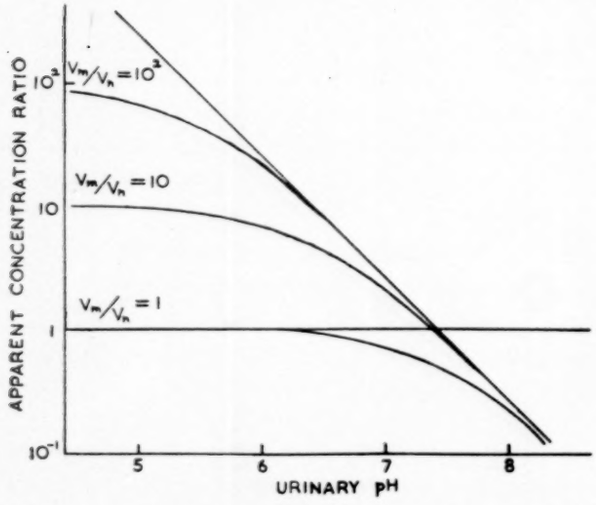


FIG. 6. The effect of incorrectly using the concentration of a diffusing base in arterial instead of renal capillary plasma when calculating the concentration ratio. The straight line represents the true ratio. The curved lines give the reduced values obtained at varying proportional flow rates of the two fluid phases. The actual value of V_m/V_n for the normal human kidney is 80, and for the dog's stomach is 40. To simplify calculations it is assumed that the base is not bound to plasma protein and is present in erythrocytes in the same concentration as in plasma.

$$V_m.C_a = V_m.C_{cap} + b.V_n.R.C_{cap}$$

but $R_a = \frac{R.C_{cap}}{C_a}$, and therefore $C_{cap} = \frac{R_a.C_a}{R}$

Substituting for C_{cap} and dividing throughout by C_a:

$$V_m = \frac{V_m.R_a}{R} + b.V_n.R_a$$

Multiplying by $\frac{R}{V_n}$:

$$R.\frac{V_m}{V_n} = R_a.\frac{V_m}{V_n} + b.R.R_a$$

Whence $$\frac{V_m}{V_n} = \frac{b.R.R_a}{(R - R_a)}$$

The effect of incorrectly relating concentration ratios to arterial rather than to capillary plasma is shown in Figure 6. The true concentration ratio of the diffusing base is represented by the straight line, log R being inversely proportional to urinary pH. Apparent concentration ratios are shown for values of V_m/V_n of unity, 10, and 100. The relationship between log R_a and urinary pH is curvilinear, and at low pH values R_a approaches a limiting value equal to $\frac{V_m}{b.V_n}$. In the kidney V_m/V_n must be approximately equal to the ratio between the renal blood flow and the rate of urine flow in full water diuresis. This ratio is about 80 in the normal human.

In the kidney, knowledge of renal haemodynamics allows an approximate prediction of R_a. In contrast, in the stomach actual measurement of R_a can be used to define gastric haemodynamics. The apparent concentration ratio between gastric juice and total arterial plasma base ($b.R_a$) is about 40 for a large number of organic bases and never exceeds this figure. (Table II.) Therefore the blood flow through the gastric mucosa must be forty times the rate of flow of gastric juice under full histamine stimulation. Since the gastric venous blood contains about one-third the original concentration of weak base [*6*], the total gastric blood flow is sixty times that of the maximal rate of secretion of gastric juice. One-third of this is distributed to gastric muscle and connective tissue and is therefore unavailable for diffusion into the gastric juice. The maximum rate of secretion of gastric juice in a dog weighing 20 kg. is of the order of 1.2 ml./minute [*14,15*]. The total portal blood flow in a dog of this size is about 480 ml./minute [*16*]. Therefore the gastric blood flow accounts for about 15 per cent of the total portal flow.

The Diffusion of Weak Bases and Acids Into Gastric and Pancreatic Juice. Several groups of workers [*17–21*] have studied, by means of gastric pouches in dogs, the diffusion of intravenously injected dyes into acid gastric juice, and are in general agreement as to which are concentrated in the stomach. Only basic dyes diffuse into the juice, whilst acidic dyes often cannot be detected therein. Maximum concentration ratios of basic dyes between juice and arterial plasma are of the order of forty times [*17,19*]. In contrast, Ingraham and Visscher [*19*] found that only acidic dyes diffused into alkaline pancreatic juice. Since the pH difference between pancreatic juice and plasma is less extreme, lower ratios than those of gastric juice were obtained.

Similar observations have been made with various drugs. The weak bases, α-acetylmethadol [*22*] and dromoran [*23*], were found to behave similarly to the basic dyes. Recently Shore et al. [*6*] have published a detailed study of diffusion of drugs into gastric juice obtained from Heidenhain pouches in dogs under maximal secretory stimulus from continuous intravenous infusions of histamine. (Table II.) It is seen that relatively strong acids of pK_a below 5 are not detectable in the juice, whilst weaker acids are present in lower concentration than arterial plasma, approximating to the theoretical values of Figure 2. Very weak bases, e.g. acetanilide, theophylline and antipyrine, of pK_a below 2, are present at concentrations higher than arterial plasma, again agreeing well with the theoretical values of Figure 1. Stronger bases usually show a concentration ratio between juice and arterial plasma of about 40 times. As previously stated, this is much less than the theoretical maximal ratio which rises to 10^6 at pK_a values above 8.0. (Fig. 1.) The behaviour of aniline ($pK_a = 5.0$) was studied in more detail, as simultaneous observations were made of the concentrations in juice and both arterial and gastric venous blood. At the concentration used, 32.5 per cent of the aniline in arterial blood was free in plasma, 11 per cent was bound to plasma proteins, and 56.5 per cent was within the erythrocytes. The gastric venous blood contained only 36 per cent of the amount of aniline in arterial blood, showing that 64 per cent had diffused into acid gastric juice. This proves that the diffusion of aniline across the erythrocytic membrane is so rapid that the intracellular component is freely available for further diffusion from plasma into gastric juice. As previously described, the limiting effect of measuring the apparent concentration ratio (R_a) instead of R is the reason for the reduction of the theoretical maximal concentration ratio of 10^4 to the observed value of 40.

It is seen from Table II that tolazoline ($pK_a = 10.3$) is an exception, as the observed concentration ratio was below 40. At this high pK_a value limitation of the concentration ratio by permeability to the ionized component becomes especially important. The observed value could be fully explained by the assumption that K_U/K_I for tolazoline is 10^4. In contrast, Stone et al. [*24*], using mecamylamine, a base with a still higher pK_a of 11.3, found a concentration ratio of 20 to 40. This high ratio could occur only if there is almost complete impermeability to ionized mecamylamine. The permeability ratio (K_U/K_I) must be above 10^5, an estimate similar to that derived from diffusion of the same base across renal tubule cells.

Non-ionic diffusion is of practical importance in connection with absorption of drugs from the stomach. In general, weak acids should be rapidly absorbed, whereas absorption of weak bases should be slow. Travell [*25*] showed that various alkaloids were poorly absorbed from the stomachs of animals with a ligated pylorus provided the contents were highly acid. The effect was particularly striking in the case of strychnine, in which instance administration of excess sodium bicarbonate by mouth reduced the oral LD_{50} dose threefold. Shore et al. [*26*] found that acids, e.g. salicylic acid and acetanilide, were rapidly absorbed from the ligated rat stomach whereas relatively strong bases, e.g. quinine, were not absorbed. It has recently been shown [*27,28*] that these results are applicable to man. The rate of absorption diminishes in the order: acids, e.g. aspirin and secobarbital > very weak bases, e.g. antipyrine > stronger bases, e.g. quinine and ephedrine. Diffusion into gastric contents may also be of forensic importance. It is useful to analyse stomach contents for certain drugs even if they have been given by injection, but it would be unwise to predict blood levels from the result obtained.

Ammonia Excretion. Ammonia is the only naturally occurring substance known to be excreted by a diffusion mechanism. Its excretion differs from that of the organic bases discussed later, as urinary ammonia is entirely produced by the renal tubule cells and is not derived from arterial blood. Conway [*29*] in fact considers

that mammalian arterial blood contains no free ammonia. The ammonia concentration is highest within the cells, and diffusion occurs both into the fluid within the tubular lumen and into renal capillary blood.

Nash and Benedict [*30*] showed that blood obtained from the renal vein contained more ammonia than arterial blood, and suggested that urinary ammonia must be synthesized by the kidneys. They erroneously considered urea to be the most probable source, but realised that their data could also be explained by deamination of amino acids. Krebs [*31,32*] showed that kidney tissue was capable of oxidative deamination of both L- and D-amino acids, different enzyme systems being concerned in the case of each isomer. He considered that urinary ammonia could possibly be derived from blood amino acids. Van Slyke et al. [*33*] measured renal arteriovenous concentration differences (in the dog) of many possible ammonia precursors. They conclusively showed that no urinary ammonia was derived from urea, that 60 per cent or more was formed from the amide nitrogen of glutamine, and the remainder from the α-amino nitrogen of various plasma amino acids. Since then glutamine has been regarded as the major source of urinary ammonia, but similar observations in species other than the dog are meagre and inconclusive.

Ammonia excretion is dependent on the two separate processes of rate of production within the tubule cells and the rate of diffusion into the fluid within the renal tubular lumen. The rate of production, like other enzymatic processes, is influenced both by the concentration of substrate and by the amount of available enzyme. Bartlett et al. [*34*] found a close correlation in dogs between plasma glutamine concentration and the rate of ammonia excretion. Many workers [*35–38*] have shown that urinary and renal venous ammonia increases after administration of various amino acids or injections of protein hydrolysates. The findings in general agree with the results of Kamin and Handler [*37*] who showed that L-glutamine, L-asparagine, L- and D-alanine and L-histidine are highly effective; L-aspartic acid, glycine, L-leucine, L-methionine and L-cysteine are moderately effective; and L-glutamic acid, L-lysine and L-arginine are completely ineffective in increasing ammonia production. Lotspeich and Pitts [*36*] considered that there was good correlation between the effect on ammonia excretion, the susceptibility to oxidative deamination *in vitro*, and the degree of tubular reabsorption of each amino acid. The more extensive observations of Kamin and Handler [*37*] however suggest that this correlation is by no means absolute. Bliss [*35*] found that the D-isomers of both alanine and leucine were more active in promoting ammonia excretion in the dog than the corresponding L-form. Whilst these experiments show that urinary ammonia can be formed from amino acids, they do not prove that this is the source material under physiological conditions. The concentrations of the amino acids were well above the normal level, and in many cases the unnatural D- or DL-form of the amino acid was used.

Variation in tubular enzyme concentration is of greater physiological importance than change in the availability of substrate. Four separate enzyme systems may possibly be of importance in renal ammonia synthesis: glutaminase, L-amino acid oxidase, glycine oxidase and transaminases. D-amino acid oxidase is obviously of no physiological importance in this respect. Davies and Yudkin [*39,40*] showed that renal glutaminase, L-amino acid oxidase and glycine oxidase, but not transaminases, were increased by chronic ammonium chloride acidosis in the rat and were decreased by chronic alkalosis produced by sodium bicarbonate administration. This gradual increase of renal tubular enzymes accounts for the slow rise of ammonia production described by many workers [*41–46*] during the first few days of metabolic acidosis. Sartorius et al. [*43*] found that urinary ammonia was still increasing on the fifth day of ammonium chloride acidosis, and Wood [*46*] showed there was a gradual increase throughout the first eight days of acidosis. A similar rise of renal glutaminase and L-amino acid oxidase has been shown to occur in potassium depletion [*47*] and after prolonged administration of acetazoleamide [*48*] in the rat. The single effective stimulus may be an increased acidity of renal tubular cells. Potassium depletion causes a fall of intracellular pH in slices of renal cortex [*49*]. Acetazoleamide produces an extracellular acidosis with fall of plasma pH, and a total body acidosis from loss of bicarbonate in the urine [*50*], but its effect on intracellular pH is less certain. The effect of acetazoleamide on ammonia synthesis varies in different species. Thus there is no evidence of a delayed rise of ammonia excretion after prolonged acetazoleamide therapy in man or the dog. Both ammonia excretion and

urinary pH return to control levels despite continued systemic acidosis [*51*]. This suggests that in man and the dog acetazoleamide acidosis is not associated with adaptive changes in renal tubular glutaminase similar to those occurring in ammonium chloride acidosis or potassium depletion.

Although earlier workers [*52–54*] had demonstrated an inverse relationship between ammonia excretion and urinary pH, Ferguson [*55*] first showed that this depended entirely upon urinary pH changes rather than on variation in systemic acid-base balance. He found that after administration of the carbonic anhydrase inhibitor, p-sulfamylbenzoate, to rats there was an immediate fall in ammonia excretion quantitatively similar to that in sodium bicarbonate alkalosis. Robinson [*56*] showed that similar effects could occur in rat kidney slices. Diffusion of ammonia into the incubation fluid was inversely related to the external pH. In man and the dog [*7,45,57,58*] there is an inverse linear relationship between the logarithm of the rate of ammonia output and the urinary pH. Clarke et al. [*45*] found a statistically significant deviation from linear regression between ammonia excretion and urinary pH, but the data were consistent with a linear relation if log ammonia excretion was used. The regression coefficient, which defines the rate of change of log ammonia excretion per unit fall in urinary pH, is minus 0.30 both in the dog and in man [*7,45*]. This closely agrees with the value found for other weak bases excreted by a diffusion process [*7*].

In summary, two separate factors influence ammonia output, rate of production in the tubule cells and rate of diffusion from the cells into the intratubular fluid. The former is chiefly dependent on changes in systemic acid-base balance, whilst the latter is governed by the physicochemical laws previously described. Changes in diffusion instantaneously follow variation in urinary pH, but the rate of production is secondary to slower adaptive processes which vary in different species. Adaptive changes are more rapid in the rat [*59*] than in man and the dog. In the rat there is often considerable deviation from the simple relationship readily demonstrable in short-term experiments in man and in the dog, between log ammonia excretion and urinary pH. Leonard and Orloff [*59*] found that in rats changes related to systemic acid-base balance are often more important than urinary pH as determinants of ammonia excretion. In particular, the regression coefficient relating log ammonia excretion to urinary pH is variable in this species, values ranging from -0.2 to -0.7. The data are consistent with the diffusion theory, which allows a possible range from 0 to -1.0. There are still, however, many unexplained phenomena relating to ammonia output. The adaptive changes in the acidotic rat may precede any detectable rise of renal glutaminase [*59*]. In the guinea pig ammonia output rises with both acidification and alkalinization of the urine from a minimum value at pH 7.5 [*60*]. Renal glutaminase rises with either stimulus, alkalosis being more effective than acidosis in this species [*61*]. In the alligator the urine is usually alkaline and contains large amounts of ammonia [*62*]. Here ammonia appears to be more related to retention of body cations and to water excretion rather than to control of acid-base balance.

Diffusion Mechanisms in the Renal Excretion of Other Weak Bases and Acids. Non-ionic diffusion is less generally applicable to renal excretion than to diffusion into acid gastric juice. Only a few weak bases and acids are known to be excreted by a diffusion mechanism. (Table I.) A weak base or acid must have certain unusual properties before excretion by diffusion can occur. The rate of diffusion of the unionized component across the cell membrane must be extremely rapid or otherwise high concentration gradients cannot occur. (Fig. 5.) In contrast, the tubule cells must be relatively impermeable to the ionized fraction since a high ratio of the two permeability coefficients (K_U/K_I) is essential. (Figs. 3 and 4.) In addition, the pK_a of the weak base or acid should be within a favourable range. (Fig. 4.) These limitations apply less to diffusion into gastric juice because of the high theoretical maximum concentration ratio of 10^6. (Fig. 1.) This maximal ratio is only 10^3 in the case of renal excretion of weak bases (Fig. 1), and 10 for weak acids. (Fig. 2.) The various factors limiting the concentration ratio are therefore more liable to overwhelm the effect in the kidney than in the stomach. In addition, the kidney excretes many weak acids and bases by specific transport mechanisms which may mask excretion by a diffusion process.

The weak acids and bases discussed in this section have many properties in common. They are almost all partially bound to plasma proteins. They rapidly diffuse both into erythrocytes and into the general intracellular compartment.

The weak bases often combine with tissue proteins, and therefore the concentration in various organs may be much higher than in plasma. They are all partially metabolized in the body and only a fraction of the unchanged drug is recoverable in the urine. As previously described, the concentration ratios between intratubular fluid and the non-protein bound fraction of the substance in renal capillary plasma cannot be directly measured. Proof of non-ionic diffusion therefore rests on the demonstration that a change in urinary pH has a profound effect on the renal clearance of the compound. The possibility that systemic acid-base balance rather than urinary pH is the determining factor in influencing the clearance must be excluded. In the case of some weak acids, e.g. citric, α-ketoglutaric and gentisic acids, and certain sulfonamides, a change in systemic acid-base balance alters the capacity of the renal tubule cells to reabsorb the acid from the glomerular filtrate. Possibly the actual determinant is the intracellular pH of the tubule cell. A useful method of differentiation from a diffusion mechanism is a comparison of the effects of three different methods of alkalinization of the urine. Small doses of sodium bicarbonate produce a maximal urinary pH but have only a slight effect on systemic acid-base balance. Acetazoleamide rapidly produces an alkaline urine which is associated with systemic acidosis. Hyperventilation alkalinizes the urine less effectively but causes considerable systemic alkalosis. In excretion by non-ionic diffusion the clearance should be influenced only by the urinary pH, and not by the diverse effects on total body acid-base balance.

In cases in which systemic acid-base balance is the factor influencing the excretion rate of a weak acid, alkalosis diminishes tubular reabsorption and therefore the clearance approaches that of inulin. In contrast, non-ionic diffusion is a purely tubular process and is therefore unrelated to the glomerular filtration rate. The clearance may be lower than that of inulin at one extreme of urinary pH and considerably higher at the other. (Table 1.) Since excretion is determined by concentration ratios, there should be no limiting tubular maximal reabsorptive or secretory capacity. The clearance at any particular urinary pH is constant whatever the plasma level. This has been experimentally demonstrated to date only in the case of mecamylamine [*63*].

In previous descriptions of clearances involving non-ionic diffusion, confusion has occurred because of careless definition of renal clearance when complicated by protein binding of the substance and diffusion into erythrocytes. When a compound is excreted by glomerular filtration and tubular reabsorption, the clearance should be calculated from the non-protein bound fraction in plasma. The process of glomerular filtration does not change the equilibrium state of the three components of the substance in the blood. In the case of a substance with a clearance higher than the glomerular filtration rate, involving tubular secretion, the total plasma content is used in calculation of the clearance. Protein binding does not make the bound fraction unavailable for tubular secretion because, as fast as the concentration of the non-bound fraction is reduced, there is immediate dissociation of the protein complex. Much more phenol red is secreted into the tubular lumen than is free in plasma [*64,65*]. Diodrast and p-aminohippurate are virtually completely cleared from plasma despite there being a considerable fraction bound to plasma protein [*8*].

Participation of the erythrocytic fraction in renal clearance has received less previous attention. Phenol red does not enter the red cell *in vitro* [*66*]. Diodrast and PAH enter the dog erythrocyte very slowly *in vitro* but much more rapidly *in vivo* [*67–69*]. Diodrast behaves similarly in man [*69,70*], but PAH does not enter human red cells either *in vitro* or *in vivo* [*69,71*]. Deformation of erythrocytes within capillaries possibly enhances the penetration of these substances *in vivo*. Since the PAH and diodrast clearances are identical both in man and the dog [*69*], it is usually assumed that the erythrocytic fraction of both diodrast and PAH is unavailable for tubular secretion. Diffusion of these substances across the erythrocytic membrane is slow [*72*], the amount diffusing during the short renal blood transit time being negligible. In contrast, the substances excreted by non-ionic diffusion cross the erythrocytic membrane very rapidly and the cellular fraction in the blood is therefore available for excretion. The experiments of Shore et al. [*6*] showed that the erythrocytic fraction of aniline diffused freely into acid gastric juice. Similar participation of the red cell fraction of a weak base diffusing into acid urine would be proved if the clearance could be shown to exceed the simultaneous PAH clearance. Baer et al. [*63*] have recorded whole

plasma clearances of mecamylamine in the dog which are 1.13 times that of PAH. The plasma extraction ratio was only 0.8 times that of PAH, which would be expected if a proportion of the excreted drug had been derived from the red cell fraction. The interpretation is obviously complicated by the possibility of pretubular deviation of red cells by a "skimming" mechanism [*9–11*]. The results, however, suggest that a significant fraction of erythrocytes actually perfuse the renal tubules.

The meaning of the term "clearance" in relation to substances excreted by non-ionic diffusion is therefore less well defined than when applied to substances excreted by glomerular filtration with subsequent tubular reabsorption or secretion. To avoid confusion the method of calculation of the clearance should be given, and preferably the degree of protein binding and the concentration of drug in the erythrocytes should also be reported. The ratio of the three blood components varies with different total blood concentration [*13*].

The urinary pH determines the rate of excretion of a weak acid or base eliminated by a diffusion process, but not the final urinary concentration of the substance. This proves that the site of diffusion in the nephron is proximal to that of the final adjustment of urinary minute volume by water reabsorption. The most distal portion of the tubule must be almost completely impermeable to both the ionized and the unionized components of weak acids and bases, otherwise there would be diffusion outwards with the reabsorbed water and the clearance of the weak acid or base would be reduced by oliguria. Excretion of ammonia [*7*] and of free salicylate [*73*] have been shown to be almost independent of rate of urine flow. Phenobarbital [*74*] is exceptional as the clearance is significantly reduced by oliguria. Osmotic diuresis has a greater influence on non-ionic diffusion than water diuresis. Ammonia excretion [*7*] and salicylate clearance [*73*] are both considerably increased by mannitol infusions. Osmotic diuresis increases the rate of flow of the intratubular fluid at the diffusion site, and therefore more weak base or acid diffuses into the tubular lumen before equilibrium of the unionized fraction is reached. Limiting factors may reduce the clearance below that theoretically expected from the increase of the osmotic load. Macpherson et al. [*73*] found that the free salicylate clearance was doubled by an infusion of mannitol which increased the excreted osmotic load fourfold. Increasing the rate of flow within the tubules reduces equilibration of the unionized fraction, and therefore rate limitation of the diffusion process becomes of greater importance.

Chambers and Kempton [*75,76*] first clearly showed the fundamental differences between excretion by a diffusion mechanism and excretion involving a tubular transport system. They compared the excretion of neutral red and phenol red in the frog. The dyes were infused into the renal portal vein, and simultaneously either ammonium chloride or sodium bicarbonate solution was injected into the aorta. Neutral red was concentrated in acid urine but not in alkaline urine. The excretion of phenol red, which involves an active renal tubular transport system, was unaffected by changes in acid-base balance. Administration of cyanide or urethane depressed the excretion of phenol red but had no effect on the physicochemical diffusion process involved in the excretion of neutral red.

Jailer et al. [*77*] showed that the excretion in man of the antimalarial drugs, quinacrine, chloroquine and santoquine, was higher in acid than in alkaline urine. The excretion of quinacrine and other amino-acridine derivatives has been investigated in more detail by Orloff and Berliner [*7*] in the dog, and by Leonard and Orloff [*59*] in the rat. In acid urine, log clearance is inversely and linearly related to urinary pH, d log clearance/d pH being about -0.3. In more alkaline urine, when the excretion rate is low, d log clearance/d pH approaches the limiting value of -1.0. This is the expected result if there were no limiting factors reducing the theoretical maximal concentration ratio. In acid urine the excretion of quinacrine exactly parallels that of ammonia. This relationship was used to estimate plasma quinacrine in field conditions in World War II when the somewhat difficult fluorimetric methods were not available [*78,79*]. Formulae were published by which plasma levels could be approximately calculated provided rates of excretion of both quinacrine and ammonia were known. The excretion of quinine in man has been claimed to be twice as rapid in acid as in alkaline urine [*80*]. Orloff and Berliner [*7*], however, consider that quinine is not excreted by a diffusion mechanism in man, but is in the dog. Cinchonine, it has also been claimed, is excreted more rapidly in acid urine in man than in alkaline urine [*81*].

The clearance of procaine in both the rabbit and the dog [*82*] is much higher in acid than in alkaline urine. Although no data on the effect of acetazoleamide on the clearance are available, there is little doubt that this is an example of a diffusion mechanism. Baer et al. [*63*] showed that the clearance of mecamylamine was greatly increased in the dog by acidification of the urine, and was depressed by alkalinization both with sodium bicarbonate and acetazoleamide. Similar results were obtained by other workers in man and the rat [*83,84*]. This is the only known example other than ammonia in which the variation of clearance of a weak base with urinary pH is of direct practical importance. About 60 per cent of ingested mecamylamine is excreted unchanged in the urine [*83*], and therefore variation in clearance influences the amount of the drug retained in the body, and the resultant pharmacological effect. Much smaller proportions of the other organic bases (Table 1) are excreted unchanged in the urine, consequently plasma levels and the amount of drug retained in the body are chiefly dependent on metabolic processes and not on the excretion rate. Haag and Larson [*85*] reported that nicotine is excreted in man four times as rapidly in acid as in alkaline urine. Further investigation is necessary before this can be accepted as an example of a diffusion process.

To date only two organic acids, salicylic acid and phenobarbital, have been shown to be excreted by a diffusion mechanism. The theoretical maximum concentration ratio of a weak acid between alkaline urine and plasma is only 10 (Fig. 2), and therefore diffusion processes are likely to be less important than in the case of weak bases. Sodium bicarbonate reduces both the toxicity and the plasma levels of salicylate [*86*]. This was shown by Smith et al. [*87*] to be due to an increased rate of excretion of free salicylate in alkaline urine. The free salicylate clearance is lower than that of inulin in acid urine, but considerably higher in alkaline urine [*88*]. Macpherson et al. [*73*], by comparing the effects of sodium bicarbonate, acetazoleamide and hyperventilation, showed that the urinary pH and not systemic acid-base balance was the determining factor in excretion, d log clearance/d pH being +0.52. Almost all ingested salicylate is excreted in the urine, but conjugates with glycine and glucuronic acid form 90 per cent of the total in acid urine [*89*]. In the higher excretion rates of alkaline urine, conjugates account for only 15 per cent of the amount ingested. The pK_a of salicylic acid is 3.0 which is below the optimum range for diffusion into alkaline urine. Calculations similar to those used in the construction of Figure 4 show that adequate concentration of salicylate could occur provided K_U/K_I is above 10^5, a figure similar to that derived for mecamylamine. Gentisic acid, with a similar pK_a, is not excreted by a diffusion mechanism. The simplest explanation is that the addition of the extra hydroxyl group in the case of gentisic acid increases permeability to the ionized component and therefore reduces K_U/K_I.

Waddell and Butler [*74*] showed that the phenobarbital clearance in man is about five times higher in alkaline than in acid urine, and that both sodium bicarbonate and acetazoleamide increased excretion. The pK_a of phenobarbital is 7.2, which is near the upper limit for concentration into alkaline urine. (Fig. 2.) Other barbiturates have a higher pK_a which, as shown in Figure 2, prevents excretion by a diffusion mechanism. Urinary excretion is of quantitative importance in elimination of barbiturates from the body only in the cases of barbital and phenobarbital. The pK_a of barbital is 7.7, which is too high for any significant degree of non-ionic diffusion to occur. The theoretical maximum concentration ratio between urine of pH 8.0 and plasma is 6.5 in the case of phenobarbital, but only 2.0 for barbital. When the pK_a of a drug is near that of plasma, small differences of pK_a can have a profound effect on the pharmacodynamics of a drug.

Alkalinization of the urine has been recommended in the treatment of both salicylate [*73*] and phenobarbital [*74*] intoxication. Other workers have reported favourably on dialysis with the artificial kidney in salicylate [*90*] and barbiturate [*91*] poisoning. Probably the ideal treatment in a severe case is a combination of both methods. Even if the clearance of a substance excreted by a diffusion mechanism is high, elimination by the kidney alone may be relatively slow. Most of the drug is within the intracellular compartment and is therefore not immediately available either for excretion by the kidney or removal by extracorporeal dialysis.

Other Types of pH Dependent Excretion. The excretion of some weak acids and bases may be influenced by changes in acid-base balance although they are not excreted by a diffusion mechanism. In the case of some weak acids, alkalosis diminishes tubular reabsorption of the

acid from the glomerular filtrate with consequent increase of clearance. Here systemic acid-base balance and not urinary pH is the determining factor. Possibly the intracellular pH of the renal tubular cell is directly concerned. The excretion of some of the acids of the Krebs cycle is the most important example of this type of excretion, and this will be discussed in the next section. The renal excretion of gentisic acid [*92*] and of certain sulfonamides, including sulfadiazine, sulfamerazine and sulfamezathine [*93,94*], is probably also of this type. Sodium bicarbonate increases the clearance of these acids but acetazoleamide has no such effect [*95*], and excretion by diffusion is thereby excluded. Hyperventilation is more potent than small doses of sodium bicarbonate [*95*]. At any given dose, the blood levels of these sulfonamides is reduced by simultaneous administration of sodium bicarbonate [*96*]. Nevertheless, alkalinization of the urine is indicated if there is oliguria, since the solubility of their acetyl products is thereby increased.

Systemic acid-base changes may also alter the distribution of weak acids and bases within the body and therefore alter their availability to the kidney. Here rate of excretion rather than renal clearance is primarily affected, since the plasma levels change. In general, acidosis tends to increase the plasma level of weak bases and decrease that of weak acids. Respiratory changes in acid-base balance are more effective than metabolic changes since the alteration of body pH is usually greater in the former. Thus breathing CO_2 at a concentration of 10 per cent or over increases plasma levels of the bases, quinacrine, chloroquine [*97*] and mecamylamine [*98*], but diminishes those of the acids, thiopental [*99*] and phenobarbital [*74*]. The available evidence suggests that, with the exception of thiopental, the distribution of the drug between the intra- and extracellular compartments is altered by changes in acid-base balance, probably by affecting intracellular protein binding. In the case of thiopental [*99*] the distribution of the drug between extracellular water and adipose tissue is influenced by change of pH. Metabolic changes in acid-base balance must be of extreme degree to produce a significant effect. Sodium bicarbonate has been reported to increase the plasma levels of phenobarbital in dogs [*74*], but the dosage used was 2.1 g./kg. body weight given during three hours. A rise of plasma concentration as a result of change in acid-base balance increases the pharmacologic effect of mecamylamine [*98*] but reduces that of phenobarbital [*74*].

Waddell and Butler [*74*] have suggested that changes in body distribution of weak acids and bases are more likely to occur if the proportionate rate of change of the diffusible unionized fraction with respect to pH is high at the pH range of plasma. Mathematically this expression equals dU/dpH divided by U, and is obtained by differentiation of the Henderson-Hasselbalch equation. For a weak acid $\frac{dU}{dpH/U}$ equals $-\log_e 10.\frac{10^{7.4}}{10^{7.4}+10^{pK_a}}$, and for a weak base, $+\log_e 10.\frac{10^{pK_a}}{10^{7.4}+10^{pK_a}}$. These expressions are large if the pK_a of an acid is below 7.4, and if the pK_a of a base is higher than 7.4. All the five drugs mentioned have a pK_a which agrees with theory, except thiopental which is a weak acid of pK_a 7.6.

The Excretion of Acids of the Krebs Cycle in Relation to Acid-base Balance. Although almost all acids of the Krebs cycle can be detected in human urine [*100,101*], information of the effects of acid-base balance on their excretion is available only in the case of citric and α-ketoglutaric acids. The physicochemical properties of these organic acids are completely unlike those excreted by a diffusion mechanism. Their molecules contain many polar groups, and thus the free acid is not very lipoid-soluble and is not freely diffusible across biological membranes. Citric acid does not diffuse freely into the erythrocyte [*102*], and tissue concentrations may be much higher than that of plasma. In fluoroacetate poisoning [*103–105*] very high tissue concentrations of citrate are found with only a slight rise of the plasma level.

Citric acid was first detected in human urine by Amberg and McClure [*106*]. Ostberg [*107*] showed that the normal range of excretion in man was 200 to 1,000 mg./day, and this was reduced in acidosis and increased in alkalosis. It has been known for many years that citrate excretion is not directly related to urine pH [*108,109*]. More recently acetazoleamide has been shown to reduce citrate excretion both in man [*45,110*] and the rat [*111*]. Hyperventilation increases citrate output more effectively than do small doses of sodium bicarbonate [*112*]. Increase of urinary citrate accounts for almost all

the rise of urinary organic acid in sodium bicarbonate alkalosis in man, but less than 50 per cent of the increase produced by hyperventilation [*112*]. In the rat, only about 50 per cent of the increase of urinary organic acid produced by injection of sodium bicarbonate is due to citrate [*113*]. Potassium depletion reduces citrate excretion both in man [*114*] and the rat [*112*]. Administration of potassium bicarbonate [*115*] or rubidium bicarbonate [*112*] greatly increases citrate output both in the potassium-deficient and in the normal rat. Under these conditions citrate may temporarily become the major urinary anion. Potassium chloride is a much less effective stimulus to citrate output, possibly because of the extracellular acidosis produced. Citrate excretion is low in primary aldosteronism [*112*], presumably because of the associated potassium deficiency. Potassium chloride increases citrate excretion in this disease, although the urinary pH becomes more acidic as the potassium deficiency is relieved.

The most plausible single explanation of these changes is that the main determinant of citrate excretion is the intracellular pH of the tubular cells, acidosis reducing and alkalosis increasing citrate output. Potassium depletion has been shown to reduce the intracellular bicarbonate of the renal tubules, with production of intracellular acidosis [*49*]. Although acetazoleamide is known to cause a fall of plasma pH and a total body acidosis from loss of bicarbonate in the urine, its effect on intracellular pH is uncertain. The "condensing enzyme" of Ochoa, which synthesises citrate from oxaloacetic acid and acetyl-coenzyme A, is extremely pH dependent [*116*], the synthesis increasing as pH rises. The changes in citrate excretion accompanying alteration in acid-base balance are not accompanied by any significant variation in plasma citrate [*112*]. Increased citrate output secondary to injection of potassium bicarbonate in the rat is, however, associated with considerable rise of renal tissue citrate [*95*]. This is comparable in degree with that produced by non-lethal injections of fluoroacetate, and occurs within twenty minutes of injection of the potassium salt. There may possibly be a dynamic equilibrium between the amount of citrate within the tubular lumen and that within the renal tubule cell. Clearances which would necessitate tubular secretion have not been reported, but there is evidence from experiments involving infusion of malate in rats [*101*] that part of the urinary citrate may be derived from that synthesised within renal tubule cells.

In human physiology, citrate excretion is also of interest in relation to the menstrual cycle and to calcium metabolism. Urinary citrate is at a maximum about the fourteenth day of the menstrual cycle, and abruptly falls to a minimum value just before menstruation occurs [*117*]. Citrate output is low in cases of ovarian insufficiency [*118*], and is increased by administration of oestrogens. The menstrual effect may, however, possibly be mediated by cyclical changes in acid-base balance. Serum sodium and bicarbonate are reduced and serum chloride increased immediately prior to menstruation [*119,120*], and alveolar pCO_2 is reduced [*121*].

Citrate output also parallels calcium excretion. It is low in hypoparathyroidism [*122*], but rises after treatment with either parathormone or calciferol. Urinary citrate increases with calcium after injection of calcium gluconate [*95*]. In acidosis, however, the effects of acidosis are predominant, and the urine may become virtually citrate-free despite continued calcium excretion. Citrate readily forms a chelate complex with calcium. Tricarboxylic acids are in general more efficient chelating agents than dicarboxylic or monocarboxylic acids [*123*]. Citric acid is a more efficient chelating agent than the closely related tricarballylic acid [*124*]. Variation of urinary citrate in parallel with calcium may possibly be due to less complete tubular reabsorption of the calcium citrate chelation complex than of either free citrate or of calcium ions. In acidosis almost all citrate is reabsorbed by the tubules, and the chelate progressively dissociates into free citrate and calcium as tubular reabsorption proceeds. Urinary citrate may be of importance in increasing the solubility of calcium in alkaline urine, and thus reducing nephrocalcinosis or calculus formation [*125*]. Urinary citrate is reduced in cases of renal calculus [*126,127*] but this may partly be due to bacterial destruction of citrate from associated urinary infection [*128*]. There are two conditions in which the association of hypocitricuria and nephrocalcinosis or stone formation is well established. In both renal tubular acidosis and after prolonged ingestion of acetazoleamide [*45,110*] hypocitricuria occurs as a result of systemic acidosis and potassium depletion. In both conditions the urine is neutral or even alkaline, and calcium excretion is often high. About half the cases of renal tubular

acidosis are complicated by nephrocalcinosis or renal calculi, and this has now been reported in at least five cases of prolonged ingestion of acetazoleamide [*110*].

Krebs [*129*] first detected α-ketoglutaric acid in human urine, and reported an excretion rate of 15 to 25 mg./day. More recent estimations using chromatographic technics [*130,131*] have given similar results. Excretion of α-ketoglutarate usually parallels urinary citrate in conditions of varying acid-base balance [*112*]. Urinary α-ketoglutarate is higher in the human female than in the male [*131*], and also shows variations similar to citrate during the menstrual cycle [*132*]. The excretion is greater during the day than at night [*131*], possibly due to diurnal variation in acid-base balance.

SUMMARY

1. Biological membranes are more permeable to the unionized fraction of some weak acids and bases than to the ionized component. This differential permeability may result in concentration of the bases in acidic fluids and of the acids in alkaline fluids. No active transport system is involved, and no energy other than that necessary to maintain a hydrogen ion gradient is required.

2. Many weak bases are concentrated into acid gastric juice, and some weak acids are present in less concentration than in plasma. Weak acids are therefore often absorbed from the stomach more rapidly than bases.

3. The excretion of a few weak acids and bases is dependent on urinary pH. The clearance of the bases is higher in acid urine and the clearance of the acids is higher in alkaline urine.

4. The quantitative aspects of the diffusion of weak bases and acids from plasma into gastric juice and urine are discussed in detail. Actual concentration ratios between these fluids and plasma are lower than the theoretical values calculated from a simple system in which it is assumed that there is complete impermeability to the ionized fraction, and that complete equilibrium is reached. It is shown that the three limiting factors of permeability to the ionized fraction, rate limitation of diffusion, and false reference of concentration ratios to arterial rather than to capillary plasma are sufficient to account for this discrepancy.

5. The excretion of ammonia and other weak bases and acids excreted by a diffusion mechanism is discussed in detail.

6. The tubular reabsorption of some weak acids is dependent on systemic acid-base balance and not on urinary pH. The use of carbonic anhydrase inhibitors in differentiation of this type of excretion from a diffusion mechanism is discussed.

7. Present knowledge of the excretion of citric and α-ketoglutaric acids is reviewed.

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Dilution and Concentration of the Urine and the Action of Antidiuretic Hormone*

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THIS review will be concerned with the renal mechanisms involved in the formation of dilute and of concentrated urine. Although these processes represent the final steps in the regulation of the osmotic pressure of the body fluids, regulatory factors in the organism as a whole, such as the control of the secretion of antidiuretic hormone (ADH), will not be considered, nor will an attempt be made to include a comprehensive review of the extensive body of information dealing with the osmotic pressure of urine. The material to be considered is that most pertinent to (1) an understanding of current views of the dilution process and the mechanism of action of ADH, and (2) the mechanism for producing a urine hypertonic to plasma. A hypothesis concerning the nature of the latter will be presented and some of its consequences considered.

Recent work indicates that only two basic tubular mechanisms are required for the production from glomerular filtrate, isosmotic with plasma (280 to 300 mOsm./kg. H_2O), of urines over the entire concentration range from the most dilute (about 50 mOsm./kg. H_2O) to the most concentrated (about 1,000 to 1,400 in man, 1,800 to 2,300 in the dog, up to 5,000 in the desert rat). These are active sodium (chloride) transport and variable membrane permeability, arranged in definite sequence along the renal tubule and oriented anatomically within the kidney as a whole. (A special role for urea in the production of the most concentrated urines will be postulated later.)

Despite virtually total lack of information concerning the details of any active transport mechanism, it is apparent that such systems do exist and that certain solutes, particularly, for present purposes, sodium ions, must be moved against gradients of their electrochemical potential. A number of models of mechanisms which could conceivably effect such movement of solutes have been proposed and although no evidence for the identity of these models with actual events is available, they do at least provide hypotheses suggesting further experimental investigation. Most of these models involve the presence within the (renal tubular) cell of carrier molecules which combine reversibly with the sodium ions [*1*].

The active transport of sodium out of the tubular lumen readily accounts for the formation of even maximally dilute urine, since sodium and its balancing anions are responsible for most of the osmotic pressure of the glomerular filtrate. However, the production of hypertonic urine clearly involves removal of water from the urine and not addition of solute to it, inasmuch as changes over the full range of urine concentration may occur with only minimal changes in the rate of solute excretion. Furthermore, it would be impossible to select any particular solute as that which could be secreted into the urine to raise its osmotic pressure since the concentration of the urine is only to a minimal extent dependent upon the nature of the solute it contains. Therefore, suggestions as to the nature of the concentrating process must include a mechanism for the active or passive movement of water itself.

When it comes to the possible active transport of water as such, the conceptual difficulties increase markedly. In solutions of the concentration of mammalian body fluids there are approximately 200 water molecules for each particle of solute. In order to transport water, the hypothetical carrier molecule, so popular in dealing with solute transport, would have to be present in much higher concentrations or turn over much more rapidly. Consequently the only type of model which has been seriously con-

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sidered as a possibility for moving water against over-all osmotic gradients is the "osmotic diffusion pump" postulated by Frank and Mayer [*2*]. In essence this mechanism is assumed to move water across cells against an osmotic gradient at its surfaces by setting up within the cell an even steeper and opposing osmotic gradient. This is accomplished by the cyclic polymerization of small molecules at one surface of the cell and depolymerization at the opposite side of the cell. However, although this mechanism is apparently sound theoretically, Brodsky and Rehm have shown [*3*] that it is thermodynamically inadequate to account for the concentrating function of the kidney if this concentrating process produces urine of the observed hypertonicity within the tubule lumen and has in the peritubular fluid the osmotic pressure of the rest of the body fluids. Actually, although it has been proposed that cells do "pump" water as such [*4*], alternative explanations based primarily on solute transport are available in each instance cited in support of the concept of water transport. With this introduction to the problem of removing water to produce hypertonic urine it is clear that it would be desirable to seek an explanation in which the primary process is one of solute transport, not requiring the movement of water against osmotic-gradients.

A hypothesis which meets this requirement is presented later in this paper. Water is presumed to move passively out of the tubular lumen along an osmotic gradient created by the active transport of sodium. Since qualitatively the same gradient is postulated to be present when a dilute urine is being excreted, water movement across the tubular epithelium must be restricted during water diuresis. This and other considerations to be presented indicate that the resistance to passive water movement of given segments of tubular epithelium can vary widely.

The nature of the cellular mechanisms which account for changes in the water permeability of tissues is by no means entirely clear but a large and varied body of evidence is available which indicates that it is by the control of these mechanisms that ADH exerts its characteristic effects. This has been demonstrated most completely in the case of frog and toad skin by Ussing and his co-workers [*5–7*]. It has been shown that posterior pituitary extracts increase the rate of movement of water across isolated membranes of the skin of frogs and toads in response to gradients of osmotic pressure. This increase in water permeability has been represented as an increase in the size of the cellular channels or pores available for the flow of water along osmotic gradients. This information as to the type of effect to be expected from pituitary antidiuretic hormone, having been acquired from simpler systems than the kidney of intact animals, lends itself to more direct interpretation. However, all evidence now available is consonant with the view that the action of ADH on urine concentration in the intact kidney is also to be explained wholly in terms of alteration of (tubular epithelial) cellular permeability to water flow.

The direct observations available from the puncture of individual nephrons provide a useful frame of reference for understanding the relationship of active sodium transport and variable membrane permeability in the diluting and concentrating processes in the intact kidney. In reviewing the processes involved in the formation of dilute urine it is advantageous to begin by considering what is known from micropuncture studies in amphibians since: (1) the amphibian is unable to form a urine hypertonic to plasma and therefore the situation is not complicated by any possible activities of the concentrating process; and (2) as a result of the work of Richards and his associates [*8*], there is available a considerable amount of information about where the various steps in the process occur. Micropuncture studies of the kidney in frogs have shown that a considerable part of the fluid is reabsorbed in the proximal convoluted tubule and that there is no change in the osmotic pressure in the course of this reabsorption [*9*]. It is clear from other data that this reabsorption involves primarily removal of sodium and the anions which balance it, since the process can proceed in the presence of non-reabsorbable solute although this requires the reduction of the concentrations of sodium and chloride in the remaining fluid well below those in the interstitial fluid outside the tubules [*10,11*].* As to the

* It has occasionally been proposed that the reabsorption of fluid from the proximal tubule may be the result of diffusion in response to the oncotic effect of the plasma proteins in the peritubular capillaries. However, so long as more than a minute fraction of the filtered solute cannot escape from the tubules, the osmotic effects of such solute would oppose those of the plasma protein and all reabsorption would cease when the osmolal concentration of non-reabsorbable solute inside the tubules became equal to the osmolal concentration of the proteins outside. The latter concentration is of the order of only 1 or 2 mOsm.

removal of water in the proximal tubule, there is no reason to believe that more is involved than diffusion of water in response to the gradient of osmotic pressure established by the removal of solute. This requires only a relatively high permeability of this segment to water.*

As the fluid moves down the distal tubule—in the amphibian, there is no loop of Henle interposed between proximal and distal segments—it becomes diluted progressively by the further removal of sodium and its balancing anions, largely chloride [*9*]. As far as this process is concerned, the only necessary difference between proximal and distal tubules is in the permeability to water. The high permeability to water in the proximal tubule must be replaced by a relatively low permeability to water in the distal tubule in order that the osmotic gradient be maintained.

To the extent that similar observations are available in the mammal (rat) the steps in the formation of a dilute urine appear to be qualitatively similar to those in the amphibian. The original studies by Walker, Bott, Oliver and MacDowell [*14*] showed the fluid in the proximal tubule to be isotonic to the plasma, but these observations were entirely in animals producing hypertonic urine. However, more recent studies by Wirz [*15*] have not only confirmed these findings but showed that the fluid in the proximal convoluted tubule remains isotonic with plasma in water diuresis (at least in the first two-thirds of the proximal tubule which is all that is accessible for puncture from the surface in the mammalian kidney). The only clear difference between the amphibian and mammal in this regard, then, is the fact that a greater fraction of the filtered fluid is reabsorbed in the proximal segment. Active reabsorption of sodium salts is clearly established [*10,11*] and psssive diffusion of water through a highly permeable tubule the simplest and most reasonable assumption.

The urine, in the mammal, then passes through the loop of Henle, a segment not present in the amphibian. There are no direct observations on the course of events in the loop, although on other grounds there are reasons for certain assumptions about what happens. These will be considered later. However, it is clear that all or part of the loop must reabsorb sodium salts and be relatively impermeable to water, because the tubule fluid reappears in the first part of the distal convoluted tubule considerably hypotonic to plasma—*whether the final urine being formed is dilute or concentrated* [*14–16*]. The latter point will be considered further in relation to the mechanism for producing a concentrated urine. However, when dilute urine is being formed, the studies of Wirz indicate that the urine enters the distal tubule already diluted and becomes progressively more dilute as it passes through the distal tubule. There is therefore reason to believe that the dilution process is quite analogous to that in the amphibian and consists of the removal of sodium salts in a segment that is relatively impermeable to water.

Since the change from dilute urine to isotonic urine, whether in amphibian or mammal, is most simply interpreted as the dissipation of an osmotic gradient, this could readily be effected by an increase in the permeability to water of those segments of the tubule which are exposed to dilute urine. This much of the effect of ADH can thus be interpreted as similar to that produced on amphibian skin and as consisting of an increase in the permeability of the distal tubule to water. Sawyer [*17,18*] has shown that in both frogs and toads the administration of ADH causes a rise in the concentration of the urine and a decrease in the urine volume by an effect not dependent on changes in glomerular filtration. These observations cannot be due to an influence on the mechanism by which the urine is made hypertonic since the mechanism is not present in these species.

The picture is complicated, in the mammal, by the fact that the concentration of the urine may, particularly in the presence of ADH, continue on up to levels well above that of plasma. However, it has been shown that the mechanism responsible for the production of hypertonicity operates in the absence of ADH [*19,20*] and it is a logical inference that the presence of ADH facilitates the formation of urine hypertonic to plasma by (1) modifying the character of the precursor fluid delivered to the site in the renal tubule at which the concentrating mecha-

* Although it has been suggested that the outward diffusion of water may fall behind the reabsorption of sodium salts (at least in the mammal) and thus result in hypotonicity of the fluid in the proximal tubule [*12,13*], there is no evidence in the direct observations that this is so [*14,15*] nor any particular theoretical consideration that requires it. This assumption was originally made to provide a function for the thin segment of the loop of Henle, that of permitting final osmotic equilibrium before the fluid entered the distal tubule. However, a more specialized function of the loop of Henle will be proposed.

nism operates, and (2) increasing the permeability to water of the renal tubule segment which separates the urine from the concentrating mechanism. The latter action will be discussed later in connection with a description of the nature of the concentrating process itself.

In support of the view that ADH facilitates the formation of hypertonic urine by modifying the character of the precursor fluid delivered to the concentrating mechanism, there is direct evidence in mammals in the studies of Wirz [*15*] which have been confirmed by Gottschalk [*16*]. In animals producing hypertonic urine the fluid collected from the first accessible point in the distal tubule is hypotonic to plasma. However, as the fluid moves down the distal tubule the concentration rises, its osmotic pressure approaching or reaching, but *not exceeding*, that of plasma. From these observations it is clear that (1) the process which leads to the formation of hypertonic urine operates at a region beyond the distal convoluted tubule, in the collecting system, as had been previously inferred from the experiments in the intact animal [*21,22*], and (2) that in the change from water diuresis to antidiuresis, the fluid delivered to this region changes from dilute to isotonic with plasma.

Thus dilute and isotonic urine are produced in the mammalian kidney by processes entirely analogous to those found in the amphibian. The ability to excrete urine hypertonic to plasma, however, is present only in birds and mammals. The nature of the mechanism by which a hypertonic urine is produced has long been one of the more poorly understood aspects of renal function. The hypothesis to be presented requires no new tubular functions. It requires only the active sodium transport and variable membrane permeability already present in Amphibia, acting in an anatomical arrangement peculiar to birds and mammals. The medulla of the kidney, in these two classes, is composed entirely of loops and collecting ducts and their supporting tissues and blood supply. With regard to the blood supply of the mammalian medulla it is important to note that it is composed entirely of postglomerular capillaries derived from the juxtamedullary glomeruli and that these capillaries arise from the cortex, run deep into the medulla, and then turn and return to the cortex. It is also notable that the presence of loops of Henle and the general organization characteristic of the renal medulla is limited to mammals and birds and that the production of hypertonic urine is also, so far as is known, confined to these two vertebrate classes.

A number of studies of the comparative anatomy and physiology of the kidney in vertebrates have directed attention to a relation between the loop of Henle and the production of hypertonic urine. For example, Peter [*23*] in 1909 noted some correlation between the maximal urine specific gravity and the relative anatomical development of the loop of Henle among a number of mammalian species. Burgess, Harvey and Marshall [*24*] lent great impetus to the idea by experiments in which they administered pituitary extract to animals in the various vertebrate classes and observed (probably incorrectly) that only in birds and mammals did it act by increasing water reabsorption by the tubules. Because the loop of Henle is present only in birds and mammals, they concluded that the antidiuretic effect of pituitary extract was due to stimulation of water reabsorption by the thin limb of the loop of Henle. This conclusion did not receive support from the studies of Walker and Oliver and their associates [*14*] who found the urine which had already traversed the loop to be dilute even though the fully elaborated urine was hypertonic. However, the observations on fluid from distal segments in the studies of Walker et al. were limited to three measurements. Subsequently the studies of osmotic diuresis conducted by Rapoport and his associates [*25*] and by Wesson and Anslow [*22*] led to the conclusion that the last step in the formation of urine more concentrated than plasma was the removal of water from previously isotonic fluid. The conclusions of Wesson and Anslow [*22*] and Smith [*21*] that the region concerned was located in the collecting system have since been confirmed by the micropuncture studies of Wirz and Gottschalk [*15,16*]. It was difficult, therefore, to assign a role in the formation of hypertonic urine to the loop of Henle.

Wirz et al. [*26*] have proposed a mechanism for producing concentrated urine which resolves this problem and embodies several other attractive features: (1) The loop of Henle is involved in the mechanism, but the actual production of hypertonic urine occurs in the collecting system. (2) The necessary active transport can be considered to involve sodium instead of water, important in view of the difficulties already noted with the presumption that water as such is transported by the cells of the collecting tubules and ducts. (3) The possible involvement of the

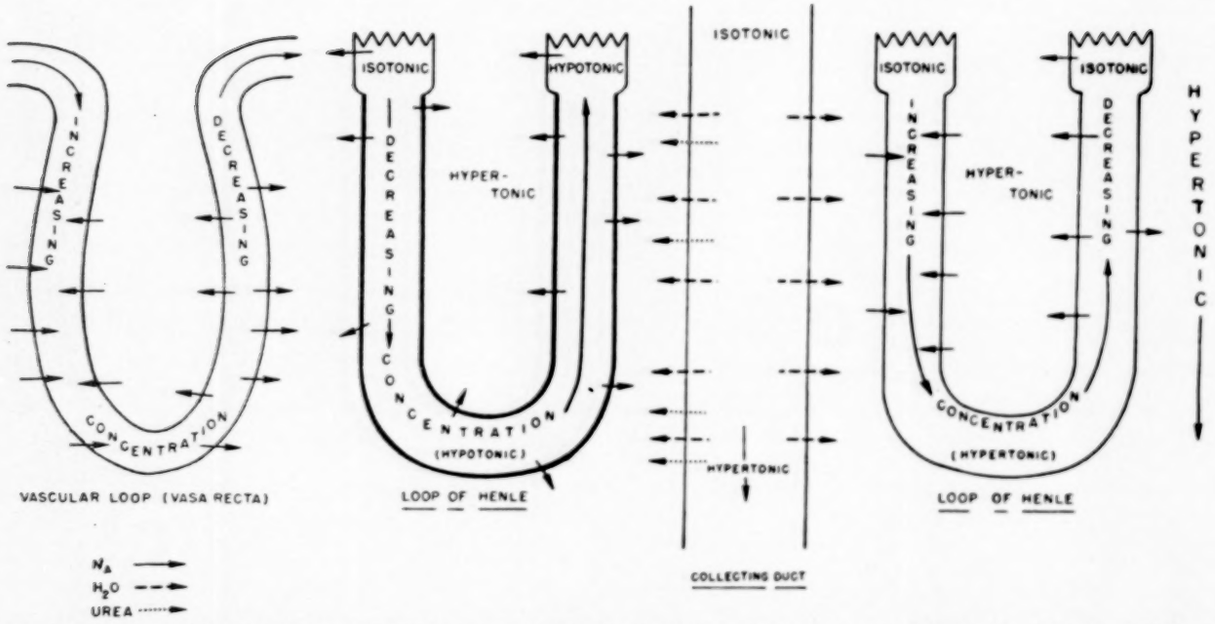


FIG. 1. Diagram of concentrating mechanism contrasting the present hypothesis (loop of Henle on left) with the views of Wirz [*26*] (loop of Henle on right). (Subsequent modifications of the Wirz hypothesis [*15*] are not illustrated.) We assume that the entire loop of Henle is water impermeable and transports sodium out of the tubular fluid into the interstitial fluid. Wirz postulated sodium transport out of the ascending limb of the loop and into the descending limb. In the original hypothesis, the loop was assumed to be permeable to water. The more recent finding of dilute fluid in the first part of the distal tubule [*15*] has led to the assumption that the thick segment of the ascending limb is water impermeable. In both views a hypertonic medullary interstitial fluid is formed; urine in the water-permeable (ADH present) collecting ducts equilibrates with the interstitial fluid and becomes concentrated. Two further features of the present hypothesis are illustrated: (1) Some urea diffuses out of the collecting ducts and increases the maximum osmolality which can be attained in the interstitial fluid. (2) High sodium salt and urea concentrations in the interstitial fluid are maintained by a countercurrent (low effective) flow in the vascular loops.

principle of countercurrent flow, important in a number of other biologic systems [*27*], is introduced.

Before describing the process suggested by Wirz it is desirable to emphasize that the loop of Henle is more than an extended length of tubule interposed between proximal and distal convolutions. Whereas the convoluted tubules are confined to the cortex, the loops run down for varying distances into the medulla, where they are closely intermixed with the collecting ducts, then turn and return to the cortex.

The function of the loop as visualized by Wirz is illustrated in Figure 1. The important process presumed to take place is transport of sodium salts from the ascending limb of the loop to the descending limb. This represents reabsorption of sodium salts from the ascending limb and secretion of sodium into the lumen in the descending limb. Because of the secretion of sodium salts into the fluid in the descending limb the concentration rises progressively as the fluid approaches the turn at the tip; having passed the turn, the fluid is progressively diluted, as it flows up the ascending limb, by the removal of the excess sodium salts which had been added. In essence, the process represents recirculation of excess sodium salts through the loop, and since the sodium salts, on the average, remain in this area longer than the water with which they enter, the osmotic pressure must be higher within the loop than in the fluid entering and leaving. The outstanding feature of this model is that the sodium need nowhere be transported over a steep gradient, since the countercurrent flow of fluid makes possible the multiplication (in the longitudinal axis) of the small gradient (between limbs of the loop). This represents an application of the multiplication principle of Hargitay and Kuhn [*28*]. Now, having established a high salt concentration, and thus a high osmotic pressure, in the lumen of the loop, one need provide only that the collecting ducts be permeable to water. Then the urine in the collecting ducts will lose water to the hypertonic surroundings (loops) and become concentrated.

However, the proposed mechanism contains an internal contradiction which to us seems insurmountable. In order for the high sodium concentration within the loops to be effective in removing water from their surroundings, the loop epithelium must be permeable to water. But if the transport of sodium from ascending to descending limb is to produce an increased sodium concentration, the loop epithelium *cannot* be permeable to water. If it were permeable to water, water would move passively with the transported sodium (as, for example, in the proximal tubule) and no concentration change could be expected. Thus permeability to water is required for the loop to operate upon its surroundings, but impermeability is necessary to establish the postulated high salt concentration. Consequently, this mechanism does not appear to be theoretically sound.

A mechanism which incorporates the attractive features of Wirz's hypothesis but is fundamentally much simpler may be postulated by modifying the proposed role of the loop. Instead of behaving as a countercurrent multiplier, the loop is viewed as a source of sodium salts and the fact that the loops dip deep into the medulla provides a means of delivering sodium salts deep into the medullary tissue. The essential steps in the formation of hypertonic urine would then be as follows:

Fluid isotonic with arterial plasma, but reduced in volume as a result of reabsorption in the proximal tubule, enters the loop of Henle. The loop is assumed to transport sodium salts from lumen to peritubular fluid as all the rest of the nephron is known to do.* No functional differentiation of ascending from descending limb is required. However, the loop epithelium is assumed to have a very low permeability to water and this permeability *is not modified by ADH*. Thus as fluid passes through the loop it becomes diluted by the removal of sodium salts; hence the dilute urine in the first part of the distal convoluted tubule. At the same time sodium salts have been left behind in excess of water in the interstitial fluid of the medulla. The dilute urine delivered to the distal tubule then loses its excess water through the water-permeable distal tubule (ADH being present) *in the cortex*. The urine, reduced in volume in the distal tubule and isotonic with arterial blood, then passes into the collecting system and re-enters the medulla, passing through the region in which the excess of sodium salts has been deposited by the loops. The collecting ducts, being permeable to water (in the presence of ADH), permit the escape of water from the urine until the osmotic pressure of the urine is equal to that of the surrounding interstitial space.

It is clear that a process of this sort could, indeed, increase the osmotic pressure of the urine above that characteristic of the body fluids outside of the renal medulla itself. However, the proposed mechanism (as well as that of Wirz) implies that the osmotic pressure of the blood as it passes through this region must also be raised to the same level as the urine. In effect, then, the blood will compete with the urine for the excess sodium salts deposited in the region by the loops. While there are no measurements of the blood flow through this part of the kidney, the possibility must be conceded that it could be quite high. For instance, even if only 5 per cent of the total renal blood flow perfused the medulla this would still, in man, amount to some 50 to 70 ml./minute. Even if the estimate is reduced to 1 per cent this would still be a flow of some 10 ml./minute and the flow of urine, some 1 to 2 ml./minute, would still be in an unfavorable position to compete. In effect, the mechanism would be called upon to concentrate large volumes of blood to produce small volumes of concentrated urine. Its efficiency, then, would be severely limited.

However, a solution to this dilemma is provided by the anatomical arrangement of the capillary circulation in the medulla which has all the necessary features of an effective countercurrent *exchanger* (a countercurrent system different in nature from the countercurrent *multiplier* proposed for the loop by Wirz [*26*]). The principle of the countercurrent exchanger has been applied to biological systems particularly by Scholander who has considered its role in the maintenance of high oxygen tensions in the swim bladder of deep sea fish [*29*] and in the regulation of heat loss from the limbs of the sloth [*30*]. An excellent review of the principle and its application can be found in Scholander's article in *The Scientific American* [*27*].

The principle is illustrated in Figure 2. Suppose an insulated pipe is fed with water at 30°c. at a flow rate of 10 ml./minute. It passes through a heat source which puts in 100 calories per minute. The water then leaves

* It has been inferred from the relatively flat nature of the loop epithelium that the loop is unlikely to be involved in performing active transport work or in maintaining large concentration or osmotic gradients. However, it is generally believed that the transport mechanisms and permeability characteristics of cells reside in the cell membranes rather than the cytoplasm. Although one may question whether or not cells of such small size contain the metabolic machinery necessary to support the transport performed at its surfaces, it is to be recalled that the squamous epithelium of frog skin is capable of transporting sodium against a concentration ratio of more than 1000 to 1. The small squamous cells of fish gills are also known to do osmotic work [*60*].

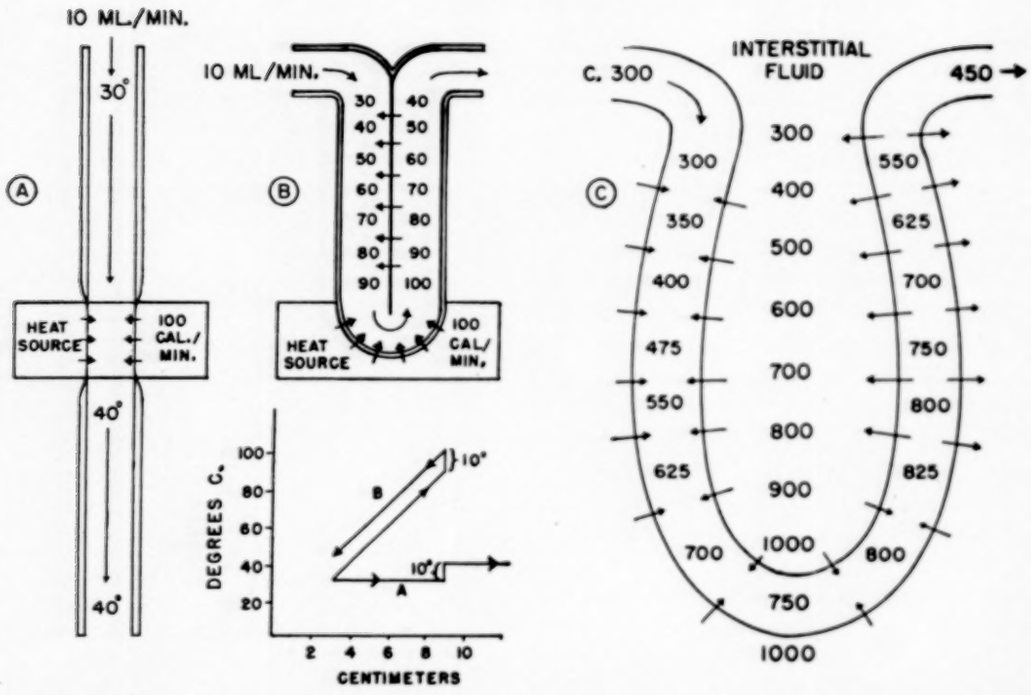


FIG. 2. Principle of the countercurrent exchanger. A and B, 100 cal./minute raise the temperature of 10 ml./minute of water 10 degrees in both A (straight flow) and B (countercurrent flow). However, since the incoming water is heated by the outgoing water in B, the *maximum temperature* attained in the countercurrent system is considerably higher than with straight flow. The graph compares the temperature along the flow tubes in each system. C, countercurrent flow as applied to the capillary loop, showing that it is not necessary for the limbs of the loop to be in direct contact. In the *hypothetical* illustration given, both limbs are in contact with the same interstitial fluid, of progressively increasing concentration. Sodium salts (arrows) at first enter the capillary blood, later partly return to the interstitial fluid; the final concentration of the blood leaving the area and the interstitial fluid depend on the various factors described in the text. Note the analogy between B, in which heat is recirculated, and C, in which sodium salts are similarly retained in an area.

the heating unit at the side opposite to that at which it entered. Since 100 calories per minute are used to heat 10 ml. of water per minute, the temperature of the water leaving the heating unit will be 10 degrees higher than that entering and the temperature in the outflow pipe will therefore be 40°C. We now introduce the factor of countercurrent flow by having the pipe emerge from the heating unit in a direction counter to that in which it entered, i.e., by having it double back upon itself. We also cause the outflow to pass in close proximity to the inflow so that heat may be exchanged between the entering and leaving streams, both still being otherwise insulated from their surroundings. In this instance the heated water coming out encounters the cool water coming in and loses some of its heat to it. Thus the entering water has its temperature increased before it enters the heating unit. The temperature increase within the heating unit must remain the same since the flow and the heat added remain constant. The temperature of the water leaving the heating unit must therefore rise above the 40°C. observed in the system with through and through flow. The process continues until a steady state is achieved which might give a temperature pattern such as that shown in Figure 2. In such a system it can be shown that the temperature at any point x from the entry of fluid is given by:

$$1) \qquad t_{xi} = t_o + \frac{\alpha H}{V^2} X$$

and

$$2) \qquad t_{xo} = t_o + \frac{H}{V} + \frac{\alpha H}{V^2} X$$

where t_{xi} and t_{xo} are the temperatures at a distance x from the start of the countercurrent system of the inflowing and outflowing fluid, respectively; t_0 is the temperature of the water as it enters the system; α is a factor related to the efficiency of the heat exchange between inflowing and outflowing fluid, H the amount of heat added to the system and V the volume flow. (Note that if the inflowing and outflowing fluid are insulated from each other α becomes 0 and the equations describe the system with through and through flow.)

As another way of looking at this system, we may consider the flow of water as intended to cool the

heated box. In this case it is clear that for the same rate of flow, the through and through system is much more effective as a cooling system than the one with the countercurrent flow. In other words, the countercurrent system *reduces the effective flow*.

The same considerations may be applied to the addition of solute rather than heat, if we allow for diffusion of solute from outflowing to inflowing blood rather than heat flow. (Fig. 2.) Actually the solute (NaCl) need not be added directly to the fluid (blood) but may be considered to be added in the region (interstitial fluid) around and between the tubes (capillaries). The capillaries need not equilibrate directly with each other since both will tend to equilibrate with the same interstitial fluid. Furthermore, although for simplicity in the model the addition was considered to occur at the tip of the countercurrent system, any arbitrary pattern of solute addition along the length of the capillary loops will give qualitatively similar results although the shape of the profile of concentration against distance will be modified.

Thus the countercurrent nature of the flow of capillary blood in the medulla provides a way in which the concentration of diffusible substances placed in this region may be increased considerably above what would be expected from the flow of blood *per se*. It thus makes it possible, in a sense, to discount the blood flow as competitive with the urine for the increased osmotic pressure produced in the medulla by the sodium salts deposited by the loops of Henle.

In order for the added sodium chloride to increase the osmotic pressure and sodium concentration in the medulla it must be added without an osmotically equivalent amount of water. To the extent that water enters the medullary interstitial fluid it will tend to carry the sodium away and keep the concentration of sodium salts from rising. Since we have assumed the loops of Henle to be negligibly permeable to water, the source of such water for the medullary interstitial fluid would be the urine in the collecting ducts. It would therefore be expected that as more water per unit time is withdrawn from the urine in the process of raising the urine osmolality (as in osmotic diuresis) the concentration of the urine finally achieved would decrease.

Also, although the countercurrent flow reduces the effective blood flow, changes in medullary blood flow would be expected to have major effects on the urine concentration since as indicated in equations 1 and 2 the concentration (temperature in equations 1 and 2) achieved would be, all other factors remaining constant, inversely proportional to the square of the blood flow.

The direction and magnitude of the effect of these various factors on the concentration achieved may be indicated as:

$$U_{Osm} = F\left(P_{Osm},\ \Delta T^{i}_{NaCl},\ \alpha,\ \frac{1}{V^{n}}\right)^{*}$$

where U_{Osm} is the osmolality of the urine,
P_{Osm} is the osmolality of the arterial plasma,
$\Delta T^{i}_{NaCl} = T^{i}_{NaCl} - T^{e}_{H_2O}P_{Osm}$,
T^{i}_{NaCl} is the net transport of sodium salts by the loops of Henle (in osmols at the concentration achieved in the medulla),
$T^{e}_{H_2O}$ is the volume of water contributed by the urine as it passes through the medulla,
α is a factor related to the effectiveness with which inflowing and outflowing blood equilibrate with their surroundings and to the length of the counter-current system,
V is the volume of blood flow through the medulla and the exponent n is a number (probably a variable) between 1 and 2, approaching 2 if the rate of net sodium transport by the loops of Henle is independent of the interstitial sodium concentration and falling toward 1 if the rate of net sodium transport is strongly dependent on the gradient from inside the lumen of the loops of Henle to the interstitial fluid.

A possibly specific position of urea in the concentrating process warrants discussion before turning to a consideration of the extent to which the hypothesis is in accord with the many observations related to the formation of concentrated urine. That a countercurrent multiplier system might be involved in the development of high urine urea concentrations has been suggested by Schmidt-Nielsen [*31*]. However, the proposed mechanism was one involving the active transport of urea by the renal tubules. The following considerations provide a mechanism by which the high urea concentrations could be achieved without active transport of urea; furthermore, the process outlined here assigns a unique position to urea in that it provides that the urea in the urine can add to the osmotic pressure of the urine without being balanced by an osmotically equivalent amount of sodium chloride in the medullary interstitial fluid.†

As is well known, the urea clearance varies with the urine flow so that the fraction of the filtered urea excreted in the urine may range

* Disregarding a series of similar factors for other diffusible substances to which the collecting ducts may be permeable (e.g., urea).

† We are indebted to Dr. Edward T. Dunham for first calling our attention to this possibility.

from some 30 per cent under ordinary conditions of antidiuresis to some 70 to 80 per cent in water diuresis. Selecting a figure for purposes of illustration, we may assume that in antidiuresis some 50 per cent of the filtered urea remains in the fluid reaching the end of the distal tubule. At this point the volume of fluid has been reduced to something of the order of 2 per cent of the glomerular filtrate. Since 50 per cent of the filtered urea is contained in 2 per cent of the filtered volume the urea concentration of the fluid entering the collecting system would be about twenty-five times that in the blood. Now if it is assumed that in the presence of ADH the collecting ducts are permeable to urea as well as water, circumstances are suitable for the collecting ducts to feed urea continuously to the medullary interstitial space. As urea diffuses into the medullary interstitial space, the concentration of urea in this area rises to considerable levels because, as in the case with the sodium salts contributed by the loop, (1) the blood flow is low or (2) the countercurrent flow of blood keeps low the effective blood flow with respect to urea, a highly diffusible substance. At the same time continued diffusion of urea from collecting duct to interstitium is maintained by the diffusion of water from collecting duct to hypertonic (NaCl) interstitial fluid. Thus, in the steady state, the urine in the collecting ducts, by contributing a fraction of the urea with which it entered, can maintain a high urea concentration in the peritubular fluid and this urea may then balance the osmotic effects of a major fraction of the urinary urea. To the extent that this is the case, then, the sodium chloride in the interstitial space is required to balance the osmotic effects of only the solutes other than urea plus that fraction of the urinary urea not balanced by urea in the interstitial space.

To this point, the hypothetical mechanism for concentrating the urine has been considered largely without reference to the many observations made in the past upon which the development of the hypothesis is based and which must be in accord with prediction if the hypothesis has validity.

A high concentration of sodium salts in the medullary interstitial fluid is a central requirement for the operation of the mechanism. The existence of high chloride concentrations in this area has been known from observations extending back for many years [*32–34*], and the finding has been repeatedly confirmed. More recently it has been found that the concentration of sodium is also high [*35–37*], in general slightly higher than that of chloride [*36*]. The more recent studies have been based upon determination of the sodium and chloride content of slices of renal medulla and papilla and have shown that the concentrations of these ions may, in the dehydrated dog, be as great as 350 and 325 mEq./L., respectively [*36*]. Since the concentrations are referred to the total water of the tissue, it is clear that the concentrations may be considerably higher in those fluids which actually contain the major part of the salt. In any case the urine makes a negligible contribution to the total, not only because in the dehydrated animal the volume of urine in the region is probably quite small, but because the sodium and chloride concentrations in the urine have generally been well below those of peripheral plasma when the studies were performed.

It has not been possible to obtain medullary interstitial fluid for direct analysis, but two types of studies by Wirz and his associates indicate that this fluid must have a high osmotic pressure. Wirz, Hargitay and Kuhn [*26*] found that the melting points of the fluids in all the medullary structures were more or less the same at any particular depth in the medulla but that the melting points indicated progressively rising osmotic pressures as the sections were obtained closer and closer to the papilla. In addition Wirz [*38*] obtained minute samples of blood from surface venous capillaries of the papilla in hamsters and found these to have essentially the same freezing point (osmotic pressure) as the hypertonic urine obtained simultaneously.

A number of workers have concluded that the cells of the renal medulla have osmotic pressures greater than that of other body cells because slices of renal medulla gain weight when immersed in solutions considerably more concentrated than plasma and even several times as concentrated as those which prevent similar swelling of kidney cortex [*35,39,40*]. The basis of the swelling of cortical slices is disputed and it is at least questionable as to whether or not valid conclusions concerning the osmotic pressure of tissues can be drawn from such observations [*41,42*]. The case of renal medulla is even more confused since, in our experience, freshly prepared slices of medulla swell even in sucrose solutions considerably hypertonic to the con-

centrated urine being produced [*36*]. Thus the question of the osmotic pressure of the cells of the medulla must, at this time, be considered open. However, there can be no question from the observations on the high salt concentrations in the area as well as the freezing point observations of Wirz et al. [*26*] that the osmotic pressure of the region as a whole is high.

As to the concentration of the fluid within the loop of Henle, the hypothesis presented here would require that it be more dilute than plasma. Actually in their melting point studies Wirz, Hargitay and Kuhn [*26*] found the contents of the loops to melt at the same time (temperature) as the fluid in other structures in the same sections. This would be in accord with the predictions of the Wirz hypothesis, but not with the modification under consideration here. However, the possibility of changes in the distribution of osmotic pressures due to diffusion within the tissue slices during the freezing and melting processes must be conceded [*43*].

The present hypothesis accounts for the dilute character of the urine in the first part of the distal convoluted tubule [*14–16*]; the unmodified Wirz hypothesis does not, although more recently Wirz has interpreted this finding as due to removal of salt without water in the thick portion of the ascending limb [*15*].

Aside from changes in tubule permeability (ADH) and the concentration of the fluid reaching the collecting system, three factors which should have marked effects on the ability of the kidney to produce concentrated urine are: (1) the amount of sodium reaching and transported by the loop; (2) the amount of water lost from the urine as it passes through the collecting ducts ($T^c_{H_2O}$), and (3) the medullary blood flow.

Regarding the amount of sodium reaching and transported by the loop, there is no direct information. Marked reductions of glomerular filtration rate might be expected to limit the delivery of sodium salts to this segment and reductions of glomerular filtration rate in excess of 30 to 35 per cent have been shown to produce marked impairment of concentrating capacity in the kidney of dogs when one kidney is compared to its undisturbed control [*36*].

Numerous observations in osmotic diuresis are in accord with the prediction that as the urine loses more and more water in the concentrating process, the concentration achieved becomes lower and lower [*10,11,22,25,44*].* It has not been possible to show that this is due to attainment of a maximal rate of performance of osmotic work [*45*] nor has the removal of a stable, fixed maximal amount of water by the concentrating process been convincingly shown [*46,47*].

To the extent that the usual assumptions about osmotic diuresis are correct* the present hypothesis would require that the urine concentration approach that of plasma as an asymptote and that simultaneously the water yielded to the concentrating mechanism ($T^c_{H_2O}$) would approach, as an asymptote, the volume which, in isotonic solution, would contain the sodium deposited in the medullary region by the loop. No sharp break in the curves relating urine concentration and urine flow to solute excretion would be expected and no true maximum value of $T^c_{H_2O}$ would be achieved. At the low end of the scale of solute excretion one would expect that each reduction of solute excretion would yield some further increase in urine concentration, although if the sodium transport by the loop were much affected by the gradient against which the sodium is transported, the further increase in concentration might be small. In the dehydrated dog excreting very concentrated urine (circ. 2,000 mOsm./kg. H_2O) and with a spontaneously low rate of solute excretion, further increases in concentration occur when solute excretion is further reduced by slight (10 to 25 per cent) reductions of glomerular filtration rate [*36*].†

Inferences concerning the effect of changing the blood flow to the renal medulla are particularly difficult to draw because there is at present no method for measuring the flow to this region of the kidney; actually there is no basis for even estimating the order of magnitude of this flow. It is possible that the failure of the hydrated subject given ADH to form urine as concentrated as can be produced by the spontaneously dehydrated subject [*48,49*] may be due to higher blood flows in the hydrated

* The calculation of water lost from the urine in all of these studies is based on the assumptions that (1) the urine reaching the concentrating mechanism is isotonic to plasma, and (2) that only water and no solute is lost once the concentrating process has begun. Although reasonable approximations, these assumptions are probably not strictly valid.

† A factor of reduced medullary blood flow cannot be excluded in such experiments.

state; certainly, the total renal blood flow is increased by hydration. Similar considerations may apply to the impairment of urine concentration in the pyrogenic reaction [*50*] in which renal blood flow is markedly increased. However, there is no evidence that changes in medullary blood flow parallel modifications of total renal blood flow.

That urine can be made more concentrated when urea is the major solute than when other solutes are excreted was first suggested by Gamble et al. [*51*]. Although the experiments from which this conclusion was drawn were open to criticism on the basis that the water intake of the experimental animals was not restricted, more recent observations suggest that the conclusion is, nevertheless, correct. Dehydrated subjects on low protein diets were found by Epstein et al. [*52*] to produce urine less concentrated than when they were on high protein diets and the administration of urea restored maximum urine osmolality to that obtained with a high protein intake. Similar observations have been made in animals.

High urea concentrations in the renal medulla have been observed by Ullrich et al. [*53*] who reported the urea concentration of the water of the renal medulla of dehydrated dogs to reach the same level as is found in the urine. The presence of high urea concentrations in the medulla has been confirmed [*36,54*] although experience in this laboratory would indicate that the concentration of urea in the water of medulla or papilla is always appreciably (20 to 30 per cent) lower than in the urine. These findings clearly indicate that there is a high urea concentration in the tissue and that the major fraction is not in the urine contained in the slice, but is probably in interstitial fluid, blood and, presumably, cells. The urea concentrations have suggested to Schmidt-Nielsen [*31*] the possibility of a countercurrent mechanism although the fact that the concentration is, on the whole, lower than that in urine is compatible with the view, presented herein, that the urine is the source of the urea in this region. As Schmidt-Nielsen has pointed out [*31*], the large amount of urea contained in the medulla and papilla may provide an explanation for the observations of Shannon on the effects of changing urine flows on urea excretion [*55*]. The elevation of urea clearance above the steady state value when urine flow is rising may be due to emptying out of the accumulations in the medulla.

Since sodium transport and relative water impermeability in the loop of Henle are assumed to be independent of the presence or absence of ADH, the present hypothesis suggests that the high sodium concentration in the medullary interstitial fluid characteristic of antidiuresis will also be present during water diuresis. The medulla presumably contains a relatively large volume of dilute urine in water diuresis and a small volume of urine of somewhat greater sodium concentration during maximum antidiuresis. It therefore does not seem valid to compare the sodium *concentration* of medullary tissue slices in different states of diuresis on the basis of microequivalents per milliliter of total tissue water, as Ullrich et al. [*35*] have done. Recalculated on the basis of microequivalents per gram of dry tissue weight their data, as well as observations in this laboratory [*36*], indicate that the *amount* of sodium in the medulla in water diuresis is 80 per cent or more of the amount found in maximum antidiuresis. The basic mechanism for the production of hypertonic urine, a high concentration of sodium salts in the medulla, is thus probably present even in water diuresis.

The present hypothesis is in accord with recent studies [*19,20*] which indicate that the concentrating mechanism functions in the absence of ADH. However, the work of Berliner and Davidson [*20*] indicates that the mechanism functions with reduced efficiency when ADH is absent. These studies were based on the hypothesis that urinary dilution is attributable to removal of sodium salts in the distal portions of the nephron and that the mechanism for producing hypertonic urine operates whether ADH is present or not. If these assumptions were correct, then reduction of the amount of sodium filtered might so reduce the amount delivered to the distal segment as to markedly limit the extent to which the urine could, even in the absence of ADH, be diluted. At the same time because of marked reduction of flow in the distal segment, even the limited permeability to water characteristic of the absence of ADH might be sufficient to permit escape from the tubule of a major part of the water rendered solute free by the removal of sodium salts. As a result a small volume of only slightly diluted urine would be delivered to the concentrating segment. Then if the concentrating mechanism operated even with reduced efficiency in the absence of ADH, it might be able to effect the removal of enough water to

render the urine hypertonic. To exclude any participation of ADH in the process, the experiments were made on dogs undergoing a maximal water diuresis and one kidney continued in this diuretic state while the experimental manipulation, consisting of reduction of the glomerular filtration rate by inflation of a cuff previously placed on the renal artery, was carried out on the other kidney. It was found that all reductions of the glomerular filtration rate reduced the extent that the urine could be diluted and that when filtration was sufficiently reduced urine hypertonic to plasma was regularly obtained. The conclusion that the mechanism for producing hypertonic urine is not dependent upon ADH is certainly warranted. However, careful consideration of the data leads to the further inference that the effect of ADH on the over-all process of forming urine hypertonic to plasma is probably not limited to modifying the character of the urine delivered to the concentrating mechanism. Urines more concentrated than plasma by margins far beyond experimental error were regularly obtained, but the observed concentrations were never greater than 475 and usually less than 400 mOsm./kg. H_2O. (Plasma osmolality in these experiments was usually about 270 mOsm./kg. H_2O.) These concentrations are far lower than the kidney in dogs is capable of producing (1,800 to 2,300 mOsm./kg. H_2O) even taking into account that the hydrated animal is unable to form a urine as concentrated as the dehydrated animal [*48,49*] and that constriction of the renal artery in the dehydrated dog, under certain conditions, decreases the maximum concentration of the urine produced [*36,56*]. On the other hand, if one were to assume that the mechanism producing a concentrated urine was able to remove from the precursor fluid only a fixed limited amount of water, this too would be an inadequate explanation for the limited concentration observed. The amount of water required to restore the observed urine to isotonicity (so-called $T^c_{H_2O}$ [*22*]) was rarely above 0.2 ml./minute and never over 0.3 ml./minute. In the intact animal given ADH this figure may rise to 5 ml./minute. Even allowing for the fact that the concentrating mechanism may, in the experiments of Berliner and Davidson, have had to remove water to bring the urine up to isotonicity before going on to higher concentrations, values *approaching* the magnitude of the normal should have at least occasionally been observed. The limited efficiency of the concentrating mechanism in the absence of ADH is most reasonably explained by the assumption that ADH increases the permeability to water of the collecting system as well as the distal convoluted tubule.

While ADH does directly promote the excretion of a maximally concentrated urine in this manner, by far the most important effect in the mammal from the quantitative point of view is exerted in the distal convoluted tubule. The increased permeability to water in this segment produced by ADH prevents the excretion of dilute urine, i.e., promotes the excretion of isotonic urine. The relative quantitative importance of these two effects can be illustrated in a person excreting enough solute to require the excretion of 2 L. of isotonic urine in twenty-four hours. If he were to undergo maximal water diuresis for this period he would excrete approximately 20 L. of urine. Therefore, if ADH were to effect a rise in urine concentration only to isotonicity with the plasma some 18 L. of water would be conserved. If, on the other hand, the same solute were to be excreted in a maximally concentrated urine, the volume would be reduced to about 500 ml, a further saving of about 1.5 L. Clearly the water saved in not putting out a dilute urine is quantitatively much more important than that conserved in forming a concentrated urine.

To simplify presentation of the renal mechanisms involved in dilution and concentration of the urine, attention has been focused on the formation of maximally dilute and maximally concentrated urine. Obviously, these are only the limits of the continuum of urine concentrations achieved by the kidney, and renal function is not to be conceived purely in terms of extreme cases or "all-or-none" changes. For example, events in the distal convoluted tubule have been described in terms of the movement of a volume of hypotonic fluid through a segment of variable permeability. It should be emphasized that neither the volume nor the tonicity of the fluid is fixed, and that the permeability of the tubule may vary over a considerable range. Even in the absence of ADH, it is unlikely that movement of water across the tubular membranes is completely restricted [*57*]. Studies with exogenous vasopressin [*58,59*] indicate that the permeability of the distal segment increases in a graded fashion with increasing levels of ADH. Whether or not any given degree of permeability is adequate to permit complete

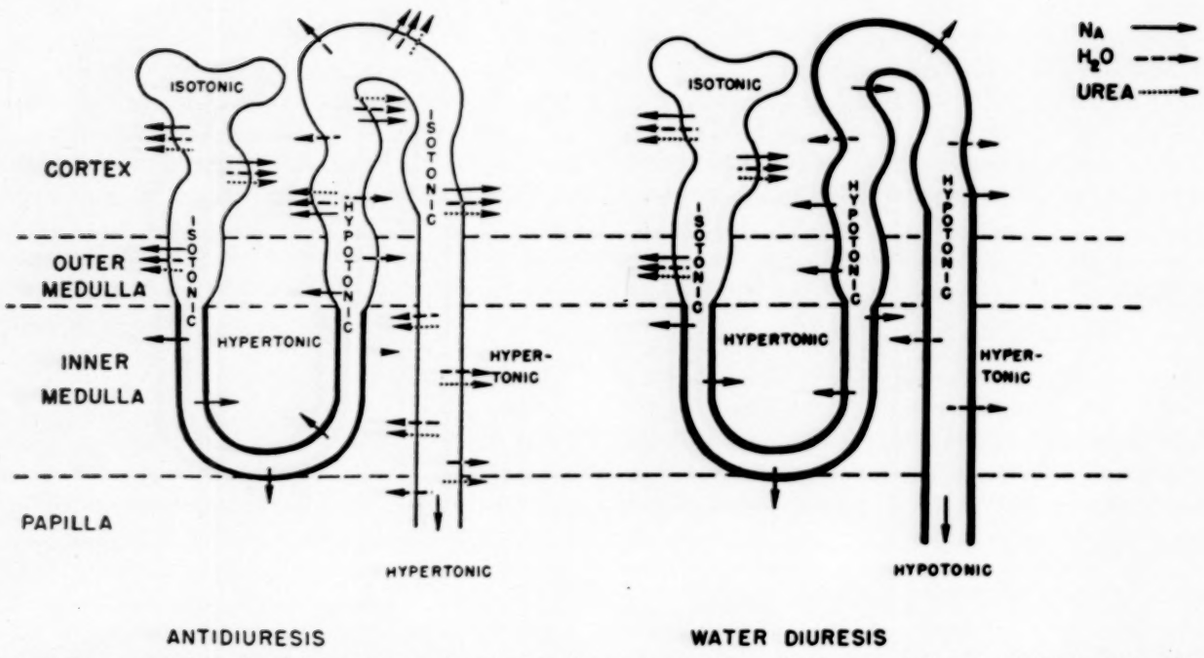


FIG. 3. Summary of renal tubular processes involved in the concentration and dilution of the urine. Water-permeable segments of the nephron are drawn with light lines, impermeable segments with heavy lines. The arrows indicate only the direction and location of sodium salt, urea and water movement; the number of arrows has no quantitative significance. This diagram is fully described in the last paragraph of the text.

osmotic equilibration of the tubular contents with the interstitial fluid depends on the amount of water movement required to achieve isotonicity, the osmotic gradient present, and the time available for equilibration. Thus a degree of ADH-induced permeability sufficient for complete equilibration when the solute load is low may not suffice in the face of increased solute (volume) inflow when the amount of water to be reabsorbed is greater and the transit time and osmotic gradient in the distal tubule are reduced [*59*].

With the reservation implicit that only the limits of the wide range of a physiologic function are described, the basic tubular events in the dilution and concentration of the urine in the mammalian kidney are summarized in Figure 3. Regardless of the tonicity of the urine being formed, the events are qualitatively the same up to the distal convoluted tubule. The glomerular filtrate is isosmotic with plasma. Active sodium (chloride) reabsorption in the water-permeable proximal convoluted segment results in reduction in volume, but no change in the osmotic pressure of the tubular fluid. Active sodium transport out of the water-*im*permeable loop of Henle, a medullary structure, dilutes the tubular contents and creates hypertonicity of the medullary interstitial fluid. In the absence of ADH (water diuresis), the distal convoluted tubule and collecting ducts are relatively impermeable to water. The hypotonicity of the tubular fluid issuing from the loop of Henle is therefore maintained, and somewhat enhanced by sodium reabsorption in the distal segment; a dilute urine is passed. When sufficient ADH is present, the distal tubule and collecting ducts become relatively water permeable. The hypotonicity of the fluid which enters the distal convoluted tubule is dissipated as water flows along the osmotic gradient into the cortical interstitial fluid. The greatly reduced volume of isosmotic fluid then re-enters the medulla via the collecting ducts. The ducts permit the escape of water from the tubular fluid until the osmotic pressure of the urine equals that of the surrounding hypertonic medullary interstitial fluid. A fraction of the urine urea passes across the collecting ducts into the interstitial fluid, where it is concentrated because of the low (effective) medullary blood flow. Thus the hypertonicity of the interstitial fluid is enhanced and maximum urine concentration is achieved.

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Some Reflections on Mechanisms of Action of Diuretics*

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DIURETICS are commonly defined as agents which increase the volume flow of urine. Such a definition is inadequate both for the clinician concerned with their use and for the physiologist concerned with their mode of action. A more useful and mechanistically precise definition is that diuretics are agents which promote the urinary excretion of sodium and either chloride or bicarbonate, i.e., those ions which are largely restricted to and which constitute the major ionic components of the extracellular fluid. Secondarily, water is excreted in proportional amounts, reducing extracellular fluid volume and body weight. This definition properly emphasizes the facts that the excretion of ions is primary, that these ions are drawn from extracellular rather than cellular stores and that increased urine volume and loss of body weight are proportional to and the osmotic consequences of loss of ions. Diuretics, as we know them today, are useful only in the treatment of edema and ascites. They have no place in the treatment of acute renal failure or of the uremia of chronic renal disease.

In edema and ascites, altered metabolism of fluid and electrolyte results in two major abnormalities of body composition. First, extracellular stores of sodium and water are increased, the major fraction accumulating in the tissue interstices and serous cavities, the minor fraction expanding the circulating blood volume [*1,2*]. Second, sodium replaces potassium within cells to a limited extent [*3*]. Therefore total body sodium increases even more than does total body water. Both abnormalities of body composition focus ones attention on what is now generally conceded to be the primary event, namely expansion of body sodium stores [*4–7*]. Secondarily, fluid is retained. In congestive heart failure, retention of fluid and electrolyte must ultimately be assigned to circulatory inadequacy; in cirrhosis, to increased portal pressure; and in nephrosis, to renal damage. However, in all instances the immediate cause of sodium and water retention is altered renal function.

It is a truism to describe this alteration in renal function as one of glomerulotubular imbalance characterized by tubular preponderance. A reduction in glomerular filtration rate without a proportional reduction in tubular reabsorptive activity must lead to retention of sodium and water. Conversely an increase in tubular reabsorptive activity without a proportional increase in glomerular filtration rate must likewise lead to retention of sodium and water. Few would dispute these general statements. However, the relative significance of reduced filtration rate and enhanced tubular reabsorption, as well as the nature of the mechanisms responsible for each, are subjects of debate. Actually both factors must play roles of essentially equal importance. Glomerular filtration rate is frequently [*8,9*] although not always [*10,11*] reduced in the edematous patient in comparison with the normal. However, whether reduced in absolute terms or not, filtration rate is certainly reduced relative to reabsorptive activity. Similarly, tubular reabsorptive activity, whether increased in absolute terms [*12–14*] or not, is certainly increased relative to filtration rate. When considering glomerulotubular balance, normal is strictly a relative term. Relative or absolute reduction in filtration rate no doubt results from constriction of afferent glomerular arterioles but whether hormonally or neurally induced is uncertain. Relative or absolute enhancement of tubular reabsorptive capacity probably results mainly from excess secretion of

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the salt retaining hormone, aldosterone [*15*]. Stimulation of sodium reabsorption by sympathetic nerve impulses [*16*] and by elevated renal venous pressure [*17*], and stimulation of fluid reabsorption by excessive secretion of antidiuretic hormone [*18*] play subsidiary roles in the pathogenesis of edema, if indeed they are of any import.

TABLE 1
A CLASSIFICATION OF DIURETICS

A. Physiological Diuretics
  1. Diuretics which increase the filtered load of sodium and/or chloride ions
    a. Colloids: albumin, P.V.P., etc.
    b. Cardiac stimulants: digitalis glycosides
    c. Vasodilators of afferent glomerular arterioles: theophylline
    d. Acidifying agents: NH_4Cl, $CaCl_2$
  2. Diuretics which more or less specifically antagonize over-reabsorption of sodium and/or chloride ions
    a. Aldosterone anti-secretory agents: amphenones
    b. Antialdosterone steroids
    c. Agents which increase velocity of tubular flow in the most distal part of the nephron: water
    d. Agents which introduce a limiting ion gradient: urea, mannitol etc.

B. Pharmacological Diuretics
  1. Diuretics which specifically inhibit transport mechanisms for sodium and/or chloride ions
    a. Xanthines: theophylline
    b. Aminouracils: aminoisometridine
    c. Mercurial diuretics: meralluride, mersalyl, etc.
    d. Chlorothiazide
  2. Diuretics which interfere with hydrogen for sodium exchange
    a. Potassium salts: KCl, $KHCO_3$, etc.
    b. Sulfonamides: acetazoleamide, chlorothiazide, etc.

Ideal therapy of edema and ascites would consist of control of the primary and initiating disease process. Within limits, bed rest and weight reduction in congestive failure, dietary management of cirrhosis and steroid therapy of the nephrotic syndrome exert their favorable effects in this manner. What are designated in Table 1 as physiological diuretics include those agents which reverse the alterations in renal function leading to fluid and electrolyte retention. They restore glomerulotubular balance, either by elevating the depressed glomerular filtration rate toward normal or by more or less specifically counteracting the enhanced tubular reabsorption induced by excessive secretion of aldosterone. On the other hand the pharmacological diuretics listed in Table 1 are agents which restore glomerulotubular balance by inhibiting specific transport mechanisms for sodium. With the exception of the digitalis glycosides* which play an important role in the management of the patient in congestive heart failure, the physiological diuretics exhibit a low order of activity. Those of demonstrated clinical efficacy are all included in the category of pharmacological diuretics. Many will object that the classification of Table 1 is forced. It is included mainly to emphasize the fact that there are a variety of approaches to diuretic therapy other than those currently most in vogue.

The problem of writing a limited review of diuretics is great: the subject is broad, the literature extensive, and a number of authors have competently treated the subject during the past few years [*19–23*]. I have therefore elected to write a selective review, considering largely mechanisms of action, and treating most extensively those subjects of greatest personal interest without regard to their immediate significance in diuretic therapy.

ANTIALDOSTERONES AS DIURETIC AGENTS

A stimulating approach and one which may point the way to diuretic therapy of the future is the observation by Hertz [*24*] that certain compounds, the amphenones, depress the secretion of aldosterone by the adrenal glands. Renold et al. [*25*] have shown that exhibition of one of these drugs to a patient with a metastasizing adrenal carcinoma led to cessation of urinary excretion of aldosterone and to a brisk diuresis of salt and water. Unfortunately, the toxicity of these drugs is such as to discourage their use even as investigative tools. Were less toxic compounds developed, they might prove to be clinically effective diuretics. A related approach is that of Kagawa [*26*] who maintains that certain synthetic steroids, 3-(3-oxo-17β-hydroxy-4-androsten-17α-al) propionic acid α-lactone (SC-5233) and its 19-nor analogue (SC-8109) act as natriuretic agents in normal animals but not in adrenalectomized animals and antagonize the action of desoxycorticosterone with respect both to sodium conservation and potassium loss. Liddle [*27*] has shown that SC-5233 induces a modest natriuresis in patients in congestive failure and an even more pronounced sodium loss in patients with

* The digitalis glycosides may act as diuretics in three ways: increase filtration rate, lower renal venous pressure, and partially inhibit the renal tubular transport of sodium.

Addison's disease who are maintained on desoxycorticosterone. Presumably these synthetic steroids compete with aldosterone and displace it from receptor sites in tubular cells where it stimulates sodium reabsorption. Could more potent analogues be synthesized, they might also prove to be clinically effective diuretics. These approaches to diuretic therapy are at present in a strictly experimental stage of development.

CARBONIC ANHYDRASE INHIBITORS AS DIURETICS

Carbonic anhydrase, first described by Roughton [*28*] in the erythrocyte, greatly accelerates the attainment of equilibrium in the reaction $CO_2 + H_2O \leftrightharpoons H_2CO_3$. The further dissociation of carbonic acid to form H^+ and HCO_3^- ions is essentially instantaneous and uninfluenced by the enzyme. Carbonic anhydrase is a metalloprotein enzyme containing zinc [*29*], with a molecular weight of about 30,000 [*30*]. Mann and Keilin [*31*] showed that its activity is strongly and specifically inhibited by sulfanilamide and other N′ unsubstituted sulfonamides. This, coupled with the observation of Davenport and Wilhelmi [*32*] that the enzyme is highly concentrated in the renal cortex, paved the way for an explanation of the metabolic acidosis which had earlier been observed to result from the administration of sulfanilamide. Höber [*33*] first suggested that renal carbonic anhydrase is in some way involved in the tubular reabsorption of bicarbonate and that sulfanilamide, by inhibiting the enzyme, causes the excretion of an alkaline urine and leads to the development of a metabolic acidosis.

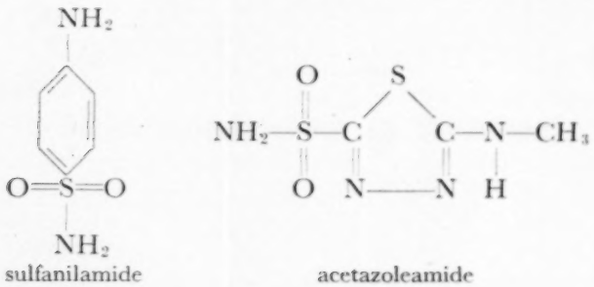
At this time two views were held as to the nature of the mechanism for acidifying the urine, both based on the premise that the acid, ultimately excreted as free titratable acid, gained access to the urine in the glomerular filtrate. According to one view, dibasic phosphate is preferentially reabsorbed from the tubular fluid, leaving monobasic phosphate to be excreted as titratable acid [*34*]. According to the other view, bicarbonate is preferentially reabsorbed, leaving carbonic acid to react with and to convert other buffer salts into titratable acid in the urine [*35*].

In 1945 Pitts and Alexander [*36*] demonstrated that acidotic dogs provided with large amounts of buffer in the form of phosphate or creatinine excreted far more hydrogen ion than was present in the glomerular filtrate. They concluded that a cellular mechanism must add acid to the glomerular filtrate as it passes through the renal tubules and suggested that the mechanism exchanges hydrogen ions, formed within tubular cells by the dissociation of carbonic acid, for sodium ions present in the tubular urine. Since the kidneys of a dog can produce titratable acid over short intervals at a rate of 6,000 cc. of N/10 acid per day, a bountiful source of H^+ ions must be available. They postulated that the role of renal carbonic anhydrase was to hydrate carbon dioxide to carbonic acid within the acidifying cells of the distal tubule and that this acid supplies hydrogen ions to the exchange mechanism. In confirmation of this thesis they noted that the inhibitor of carbonic anhydrase, sulfanilamide, markedly depressed the excretion of titratable acid.

Subsequently Pitts and Lotspeich [*37*] noted that sulfanilamide blocked a fraction of the reabsorption of bicarbonate and assigned this fraction to the distal tubule, pointing out that the exchange of hydrogen ions for the sodium ions of a solution containing bicarbonate would convert it to carbonic acid. Subsequent dehydration of the carbonic acid to CO_2 and water, and back diffusion of the CO_2 into the renal venous blood would, in effect, be equivalent to the reabsorption of bicarbonate. Roughly one-fifth of bicarbonate reabsorption was presumed to occur in the distal segment by this mechanism; four-fifths was thought to occur in the proximal segment by a specific transport mechanism for bicarbonate ion. This view is now known to be erroneous; all bicarbonate is probably reabsorbed by ion exchange, but the demonstration of this fact by Berliner [*38*] awaited the development of carbonic anhydrase inhibitors more effective than sulfanilamide. However, the fact remains that the proximal and distal mechanisms for bicarbonate reabsorption, although basically ion exchange in character and dependent on carbonic anhydrase are, at least in some respects, dissimilar, a question which will be considered in more detail later.

It now became apparent to Schwartz [*39*] that sulfanilamide might have potentialities as a diuretic. A study on patients in congestive failure demonstrated natriuresis, loss of weight, and incidentally a pronounced excretion of potassium. It was on the basis of these observations that Roblin and Clapp [*40*] synthesized a series of highly active heterocyclic sulfonamide inhibitors of carbonic anhydrase, including acetazoleamide, in the hope of finding an orally effective

diuretic. The important functional component of



sulfanilamide acetazoleamide

sulfanilamide and acetazoleamide is the unsubstituted sulfonamide radical. Figure 1 illustrates the concept of Roblin [*41*] that enzyme

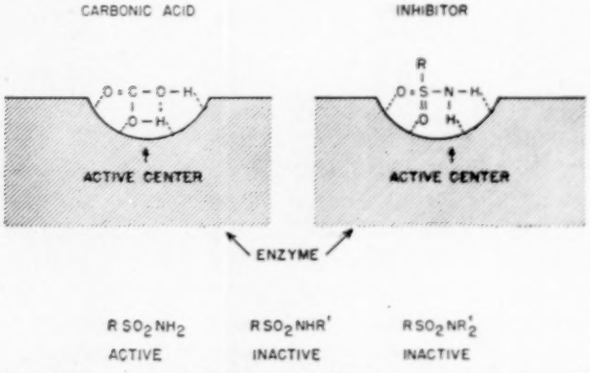


FIG. 1. Concept of Dr. R. O. Roblin of the mechanism of action of carbonic anhydrase inhibitors based on steric similarities between carbonic acid and the unsubstituted sulfonamyl group. (From SPRAGUE, J. M. Chemistry of diuretic agents. *Proc. New York Acad. Sc.* [*95*].)

inhibition results from steric similarities which cause the sulfonamide radical to bind to active sites, displacing carbonic acid.

Maren [*42*] has described the diuretic effects of acetazoleamide in normal dogs. When the drug is given in small doses of 5 to 10 mg. per kg. it promptly alkalinizes the urine. The excretion of sodium, potassium and bicarbonate are increased. The duration of action is relatively short and during the remainder of the day the losses of ions are made up from dietary intake. Under such conditions the drug exerts a constant action from day to day. However, if dosage is increased and especially if multiple doses are administered, initial ion losses are greater and, after a period, the animal becomes refractory to the drug.

Reduction in diuretic efficacy no doubt depends on a number of factors. One of the major ones, however, is the depletion of the bicarbonate stores of the body, i.e., the development of metabolic acidosis [*20*]. When the plasma bicarbonate is reduced, the filtered load decreases. Less bicarbonate is presented to the renal tubules for reabsorption and the demands on the mechanism for exchange of hydrogen ions for sodium ions are reduced. Therefore, even though the enzymatic production of carbonic acid is reduced, the uncatalyzed rate of formation may be adequate to supply the needed hydrogen ions

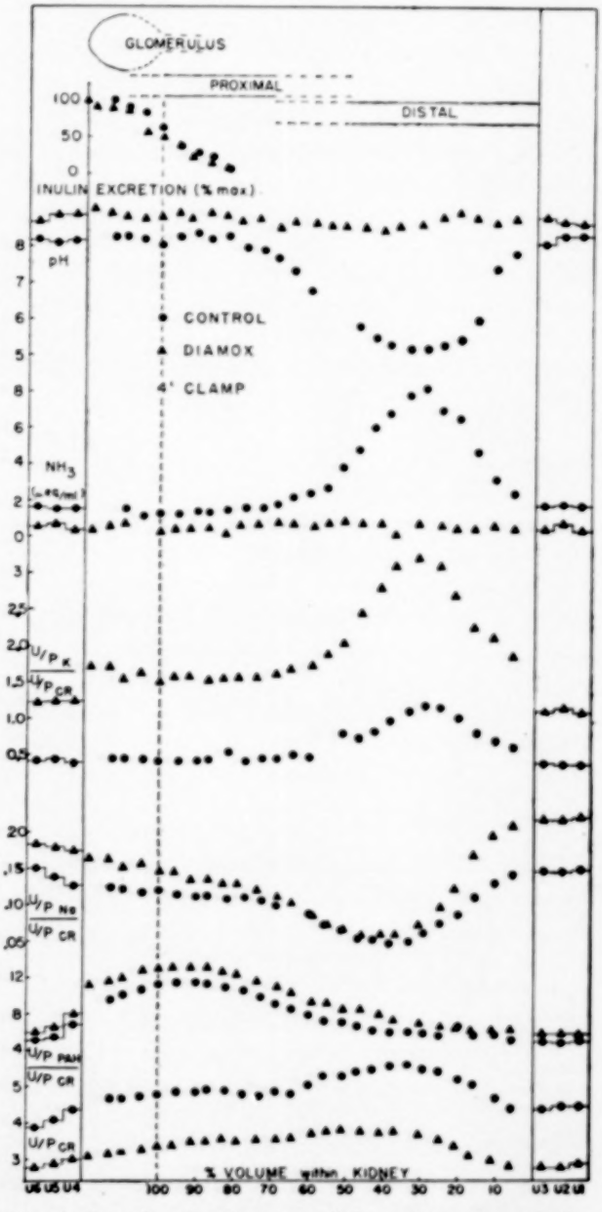


FIG. 2. Stop-flow localization of the action of acetazoleamide in inhibiting acidification of the urine, ammonia secretion and bicarbonate reabsorption and in stimulating potassium secretion in the dog. (From PITTS, R. F., GURD, R. S., KESSLER, R. H. and HIERHOLZER, K. Localization of acidification of urine, potassium and ammonia secretion and phosphate reabsorption in the nephron of the dog. *Am. J. Physiol.* [*44*].)

to the exchange mechanism. A second factor that reduces diuretic efficacy is the decrease in filtration rate which occurs in response to depletion of extracellular fluid volume [*20*]. A third factor may be stimulation of adrenal cortical activity through a receptor mechanism sensitive to extracellular fluid volume. A fourth may well be hypokalemia which is known to stimulate the distal tubular mechanism of acidification and ammonia secretion.

If one neglects the depression of filtration rate which commonly results when large doses of acetozoleamide are given intravenously, all actions of the drug can be explained in terms of a specific depression of the mechanism for exchange of hydrogen ions for sodium ions. This action, exerted on the proximal tubule, depresses reabsorption of bicarbonate. Exerted on the distal tubule, it further depresses bicarbonate reabsorption and prevents acidification of the urine. Since the urine becomes alkaline, the distal secretion of ammonia is reduced [*43*]. The mechanism for hydrogen and sodium exchange is also concerned with potassium and sodium exchange [*38*]. The over-all activity of this joint mechanism in reabsorbing sodium is only moderately affected by acetazoleamide. However, blocking of hydrogen transfer by carbonic anhydrase inhibitors facilitates potassium exchange, and exhibition of such drugs in large doses frequently causes net potassium secretion. Acetazoleamide has no significant actions on other renal transport mechanisms [*20*].

Sites of Action of Acetazoleamide. Figure 2 illustrates both the nature and the localization of these actions of acetazoleamide [*44*]. The data in Figure 2 were derived from an experiment on a dog, utilizing the "stop-flow" method of analysis of tubular function devised by Malvin, Sullivan and Wilde [*45*]. Briefly the method employed follows. One ureter was catheterized through a flank incision and an osmotic diuresis was initiated by the infusion of 20 per cent mannitol containing creatinine and p-aminohippurate. When the urine flow from the one kidney attained a value of 8 to 10 cc. per minute, the ureteral catheter was clamped for a period of four minutes. One minute before the clamp was released, inulin was given intravenously. The clamp was released and some thirty odd samples of urine were collected over the succeeding two minutes, each some 0.7 cc. in volume. The reasoning behind this experimental technic is the following. During the clamp-time, pressure builds up rapidly within the tubular system and filtration ceases. An essentially static column of fluid remains in contact with the tubular epithelium. During this prolonged period of contact, the tubular epithelium performs in exaggerated degree those operations on the static column of fluid which it normally performs on the moving column. When the clamp is released, fluid under pressure is ejected forcibly, the first samples coming from more distal parts of the nephron and later samples from more proximal parts. The final samples contain increasing amounts of inulin, a substance which serves to mark the time of appearance and to quantify the admixture of fresh glomerular filtrate.

The U/P ratio of creatinine shown at the bottom of the chart gives an indication of water reabsorption. Dividing the U/P ratios of potassium, sodium and p-aminohippurate by the U/P ratio of creatinine corrects each for water reabsorption. Such ratios of U/P ratios have the connotation of clearances of the substances in question divided by filtration rate. Thus if the U/P ratio for potassium divided by the U/P ratio for creatinine is greater than 1.0, potassium must have been added by tubular secretion. If the ratio is less than 1.0, then potassium must have been reabsorbed.

Figure 2 summarizes data obtained in two experiments on the same kidney of one dog, the control experiment performed first, and the acetazoleamide experiment immediately after. Data designated U_{1-3} on the right and U_{4-6} on the left of the diagram were obtained in conventional clearance periods immediately preceding and following the four minute clamping period.

It is evident in the control experiment (circles) that the reaction of the urine drops in the distal nephron to pH 5.0 from an average value of roughly 8.0* in the proximal segment† and in the clearance controls. The region of maximum acidification is obviously that of maximum secretion of ammonia and potassium. It is also the locus of development of the maximum

* Since it was impossible in the experimental system employed to prevent loss of CO_2, all specimens were equilibrated with air prior to measurement of pH. This exaggerates pH difference for it causes those samples which contain bicarbonate (proximal) to become more alkaline, whereas those with negligible bicarbonate (distal) do not change their reaction.

† The specimens in which the ratio $U/P_{PAH}/U/P_{Cr}$ is maximum are presumably derived from the proximal part of the nephron.

transtubular gradient for sodium. Following acetazoleamide (triangles), acidification of the urine is blocked, as is the secretion of ammonia. In contrast, the secretion of potassium is markedly enhanced while distal sodium reabsorption is essentially unchanged. This experiment

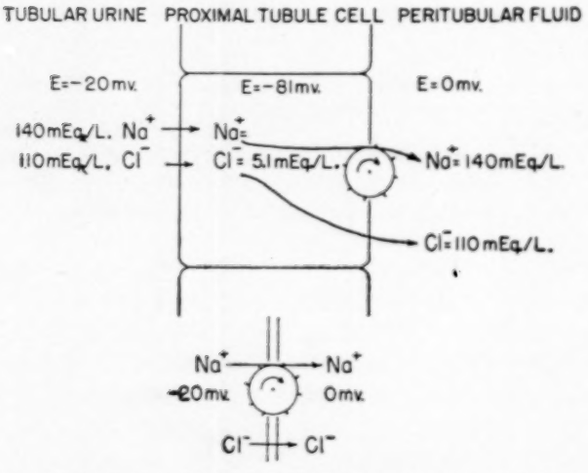


FIG. 3. Concept of a proximal tubular ion pump which would actively transport sodium ions and which would passively exchange hydrogen ions; i.e., the mechanism which, in effect, reabsorbs bicarbonate in the proximal tubule. See text for details of operation and for assumptions upon which the concept is based.

graphically portrays the reciprocal relationship between the exchange of hydrogen and potassium for sodium and emphasizes the fact that acetazoleamide, by inhibiting distal hydrogen transport, facilitates potassium transport. The higher values for $U/P_{NA}/U/P_{Cr}$ in the proximal specimens after administration of diamox® suggests that a small fraction of proximal sodium reabsorption is inhibited, i.e., that fraction associated with the unreabsorbed bicarbonate ion.

Role of Carbonic Anhydrase. Any satisfactory understanding of the action of carbonic anhydrase inhibitors as diuretics must be based on an appreciation of the role which the enzyme plays in the transport mechanism. Acidification of the urine bears a superficial resemblance to gastric acid secretion in that hydrogen ions are transferred from extracellular fluid, where they are present at low electrochemical potential, to urine or gastric juice where they are present at high electrochemical potential. Some have inferred that the transport mechanisms are basically similar and have assigned to carbonic anhydrase a role equivalent to that envisioned for it in gastric secretion, namely that of provision of a weak acid to neutralize the hydroxyl ions remaining in the cell in consequence of the active extrusion of hydrogen ions [*46,47*]. According to this view the active secretion of hydrogen ions is the important element in acidification of the urine [*20,21*]. For a number of reasons I disagree with this concept. The gastric secretory mechanism is directed toward the production of a strong acid (HCl) in high concentration (0.170N) to provide an optimum medium for gastric digestive processes. The proximal mechanism for bicarbonate reabsorption and the distal mechanism for acidification are directed toward the salvage of sodium from the tubular urine. Since hydrogen ions are potentially available in unlimited supply, they are substituted in the urine for the valuable sodium ions. Acidification of the urine and reabsorption of bicarbonate are secondary to the salvage of sodium and are not ends in themselves. The gastric secretory mechanism can establish a hydrogen ion gradient between gastric juice and blood in excess of 1,000,000:1. The proximal tubular mechanism for reabsorption of bicarbonate can establish no measurable gradient at all, i.e., the proximal urine is isohydric with blood plasma [*48*]. The distal acidifying mechanism can at best establish a gradient of 800:1, i.e., urine pH 4.6, plasma pH 7.4. It is my view that in both systems the inward transport of sodium across the tubule is the primary and active event. In the proximal system the reverse migration of hydrogen ions is passive, i.e., downhill along an electrochemical gradient. In the distal tubule a similar mechanism may be operative but it appears more likely that the outward transport of either hydrogen or potassium is linked to the inward transport of sodium by a coupled pump arrangement much like that envisioned in nerve [*49*]. In both proximal and distal systems the hydrogen ion concentration of the cell contents varies as a function of the activity of carbonic anhydrase and the pCO_2 of the body fluids [*50–52*]. Carbonic anhydrase is probably concerned with the provision of hydrogen ions to the exchange mechanisms, not with the mopping up of hydroxyl ions left in excess in the cell in consequence of the active secretion of hydrogen ions.

Nature of the Proximal Exchange Mechanism. Certain key facts are significant in developing a theory of proximal reabsorption of bicarbonate. The volume of glomerular filtrate is reduced to about one-sixth as it flows along the proximal segment [*53*]. The pH [*48*] and the sodium ion

concentration [*54,55*] do not change appreciably in the proximal segment. Therefore sodium and bicarbonate are reabsorbed in equivalent quantities and in proportion to water; hence no ionic concentration gradients of any magnitude are established. The transcellular potential varies between 60 and 90 mv. with the interior of the cell negative. In Figure 3 the value has been indicated as 81 mv. because of certain arithmetic simplifications which this figure allows in the calculations.* The average transtubular potential is 20 mv. with the tubular lumen negative to the peritubular fluid [*56*]. There exists, therefore, a net potential difference between interior of cell and lumen of 61 mv. The lumenal membrane of the cell is obviously permeable to sodium and chloride but let us assume that it is impermeable to bicarbonate. Let us further assume that the transcellular potential of 81 mv. is developed in consequence of the active extrusion of sodium from the cell into the peritubular fluid. If for any reason the net flux of sodium into the cell from the tubular urine exceeds the net flux of chloride, the potential difference between lumen and cell will be less than that between peritubular fluid and cell. This is, in fact, our view of the origin of the transtubular potential of 20 mv. This origin is implicit in the simplified diagram at the bottom of Figure 3. If the peritubular membrane of the cell is permeable to bicarbonate, that which is formed within the cell by the hydration of carbon dioxide will diffuse outward along an electrical gradient to establish an equilibrium concentration ratio of roughly 1:21 between cell contents and peritubular fluid. If the pCO_2 within the cell is essentially the same as that of the peritubular fluid, the pH of the cell contents will be roughly 6.1, i.e., the hydrogen ion concentration will be twenty-one times that of the plasma or tubular urine. The net electrical gradient of 61 mv. across the lumenal membrane is so oriented as to oppose the diffusion of hydrogen ions from cell to lumen. Were the cell pH 6.4, no net passive transfer of H^+ ions between tubular cells and lumen would occur. However, if the pH were as low as 6.1, as predicted, the concentration gradient would favor passive diffusion of hydrogen ions from cell to lumen.

A more simple way to view the situation is to consider the tubular epithelium as a single membrane separating tubular lumen and peritubular fluid. The active transport of sodium in excess of the passive transport of chloride establishes the observed potential difference of 20 mv. between lumen and peritubular fluid. The orientation of the potential is such as to favor passive diffusion of hydrogen ions from peritubular fluid to tubular urine along an electrical gradient. In any event there is no necessity to postulate a complicated redox mechanism to elevate the electrochemical potential of hydrogen ions to the fantastic heights required in gastric secretion [*46,47*].

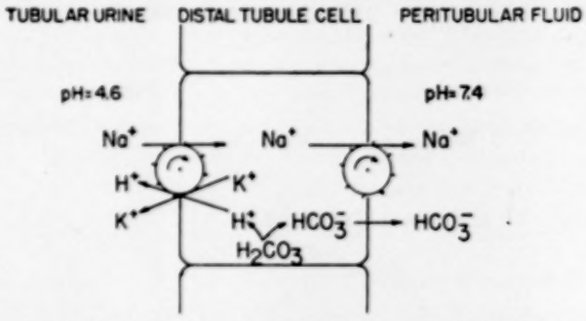


FIG. 4. Concept of a distal tubular coupled ion pump which would reabsorb sodium ions in exchange for hydrogen ions or for potassium ions. See text for details and assumptions.

A few simple considerations cast some doubt on the likelihood that passive transport of hydrogen ions can account for distal acidification of the urine. The ability to develop even the modest hydrogen ion gradient of 800:1 would demand a transtubular potential difference of about 150 mv., a value far in excess of any experimentally measured in the kidney [*56,57*].* This fact suggests that the transport of hydrogen ions may be coupled with that of sodium ions in such a way as to cause them to migrate against an appreciable electrochemical gradient, as shown in Figure 4. The well established competition between hydrogen ions and potassium ions for the distal exchange mechanism likewise suggests a coupled carrier mechanism. Obviously the affinity of the carrier for hydrogen ions must

* The calculation of concentrations which follow are based on the simplified Nernst equation:

$$E = 61 \cdot \log \frac{C_1}{C_2}$$

Choosing a value of 81 mv. as the transcellular potential difference establishes a lumen to cell potential difference of 61 mv. At 37°C. this corresponds to a 10:1 concentration difference for an ion passively distributed by electrochemical forces.

* Giebisch has, however, pointed out that the transtubular potential is greater for the distal tubule than for the proximal tubule.

far exceed its affinity for potassium ions, for the relative concentrations of these two ions species in the cell are so different. To explain competition, were either or both ions passively transported, would be extremely difficult. The role of carbonic anhydrase is, according to this view, essentially the same in the distal and in the proximal exchange mechanism, namely the provision of adequate quantities of hydrogen ions, at the relatively low electrochemical potential represented by the pCO_2 of the body fluids, to the exchange mechanism. Inhibition of carbonic anhydrase raises cell pH, blocks proximal bicarbonate reabsorption and favors potassium transport by the distal mechanism. The factor of coupled transport renders the distal mechanism somewhat less sensitive both to carbonic anhydrase inhibitors and to pCO_2 than the proximal mechanism [*50,58*]. Another factor favoring a coupled transport mechanism located specifically in the lumenal membrane of the cell is the fact that the acidifying region of the distal tubule can establish high transtubular sodium and chloride ion gradients, i.e., concentrations of only 1 or 2 mEq./L. in tubular urine.

The descriptions of the proximal mechanism of bicarbonate reabsorption and of the distal mechanism of urine acidification presented above must be considered only as reasonable speculations. Although the transtubular potential differences are in the same range grossly in the mammalian [*57*] and amphibian [*56*] kidneys, it has not been demonstrated that the pH of the tubular urine undergoes no change in the proximal segment of the mammal.

ORGANIC MERCURIAL COMPOUNDS AS DIURETICS

Although calomel was employed as a diuretic at least as early as the fifteenth century, the first use of an organic compound of mercury in the treatment of edema dates only to 1920 [*19*]. The organic mercurials as a group are by all odds the most effective of the diuretic agents presently available and constitute the standard with which other diuretics are compared.

Basic Actions of Mercurial Diuretics. For a decade after their introduction the organic mercurial diuretics were thought to exert their action by mobilizing salt and water in the tissues [*19*]. Bartram's [*59*] demonstration in 1932 of a renal action remains today a classic of simple and direct scientific proof. A minute dose of a mercurial diuretic introduced into one renal artery caused a prolonged increase in urine flow in that kidney only. A large dose caused renal shut down on the side injected and diuresis in the opposite kidney. These simple experiments demonstrated three fundamental facts concerning the action of mercurial diuretics: (1) they act directly on the kidneys; (2) they are fixed in renal tissue and exert a prolonged diuretic effect; and (3) in high concentration they are toxic and cause renal damage.

A summary of a rather sophisticated modification [*60*] of Bartram's experiment is presented in Figure 5. The two ureters of an anesthetized dog were separately catheterized and the animal was infused with creatinine to permit measurement of glomerular filtration rate. Following two control periods, shown at the left of the chart, a small dose of chlormerodrin was injected into the left renal artery. After a delay of ten minutes a brisk diuresis of sodium and water occurred which was restricted to the left kidney for some sixty to seventy-five minutes. This diuresis occurred without change in filtration rate. It must, therefore, have resulted from inhibition of tubular reabsorption of fluid and electrolyte. Uptake of the diuretic by the left kidney was not complete; some obviously escaped into the general circulation. This accounts for the accumulation of some of the diuretic in the right renal cortex, for its excretion in the urine formed by the right kidney, and for the delayed diuresis which gradually developed on the right side.

The Question of Blockade of Reabsorption of Sodium or Chloride. It has been thoroughly established [*19*] that the major and therapeutically desired action of mercurials is partial blockade of tubular reabsorption of sodium and chloride ions. A fair amount of attention has been devoted to the questions: do mercurials block chloride or sodium reabsorption primarily? If, as many claim, chloride reabsorption is specifically blocked, is natriuresis merely a consequence of the necessity for the excretion of equal numbers of cations and anions? Most would answer these questions in the affirmative for the following reasons. In mercurial diuresis the rate of excretion of chloride is commonly greater than that of sodium, the sodium deficit being made up by potassium and ammonia. With repeated courses of mercurials, loss of body chloride in excess of sodium leads to the development of a hypochloremic metabolic alkalosis. If the plasma chloride concentration drops below 90 to 95 mEq./L. and if the plasma bicarbonate concentration rises above 30 to 35 mEq./L.,

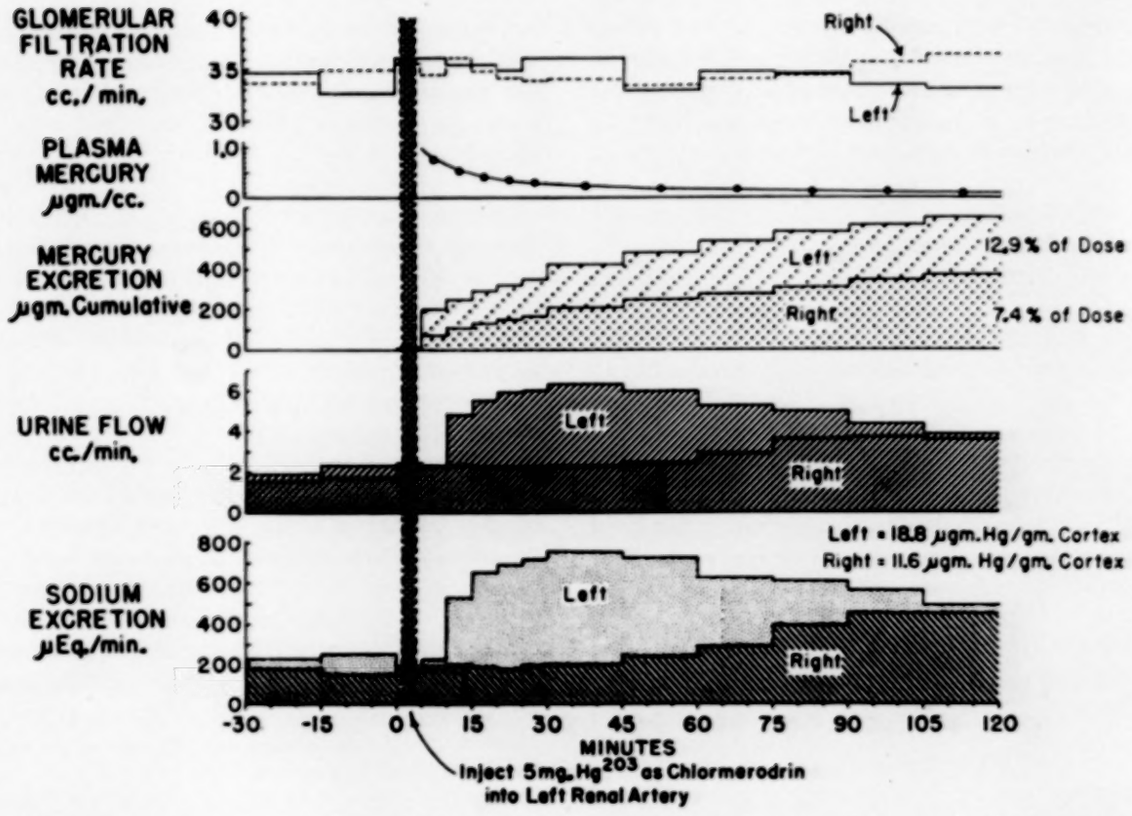


FIG. 5. Unilateral diuresis in the dog produced by the slow injection of 5 mg. Hg as chlormerodrin into the left renal artery.

mercurial diuretics become ineffective. If proper chloride levels are restored by the administration of ammonium chloride, responsiveness to mercurials returns. If hyperchloremic acidosis is induced, the diuretic activity of mercurials is markedly enhanced.

Strangely, these observations do not prove the primacy of blockade of chloride reabsorption. As Berliner [*61*] has pointed out, they are compatible with primary blockade of sodium reabsorption in the proximal tubule. If less sodium is reabsorbed proximally, more sodium and hence more chloride will be delivered into the distal tubule. If the mechanisms for exchange of hydrogen, potassium and ammonia for sodium are stimulated, as they appear to be in edematous patients, less sodium than chloride will be excreted and the sodium deficit of the urine will be made up largely by potassium and ammonia. If, as may well be true, sodium transport is active and chloride transport passive, blockade of sodium reabsorption by mercurials would be expected. However, there is really no evidence either for or against an independent active transport mechanism for chloride. If one exists, mercurials could inhibit it. I am inclined now, although not previously [*62*], to the view that sodium reabsorption is active, chloride reabsorption passive.

Effects on Water Reabsorption. It seems fairly certain that increased urine volume following mercurial diuretics is the osmotic consequence of inhibition of reabsorption of ions and is not due to any direct interference with the mechanism which transports water against an osmotic gradient; i.e., mercurials do not block the mechanism for concentrating the urine, they merely render it less effective by increasing the osmotic load of salt in the urine. Thus Brodsky et al. [*63*] have observed identical *urine flow–urinary osmotic load* relationships in hydropenic dogs infused with hypertonic mannitol or given mercurial diuretics. Capps et al. [*64*] have noted that in maximally hydrated subjects, given mercurial diuretics, urine osmolarity increases and urine flow decreases when they are infused with antidiuretic hormone, much as in control subjects. The renal mechanism which responds to endog-

enous or exogenous antidiuretic hormone seems unaffected by mercurial diuretics.

Site of Action within the Nephron. The site of action of mercurial diuretics within the nephron has long been a matter of controversy. It seems evident that mercurials can block only a fraction

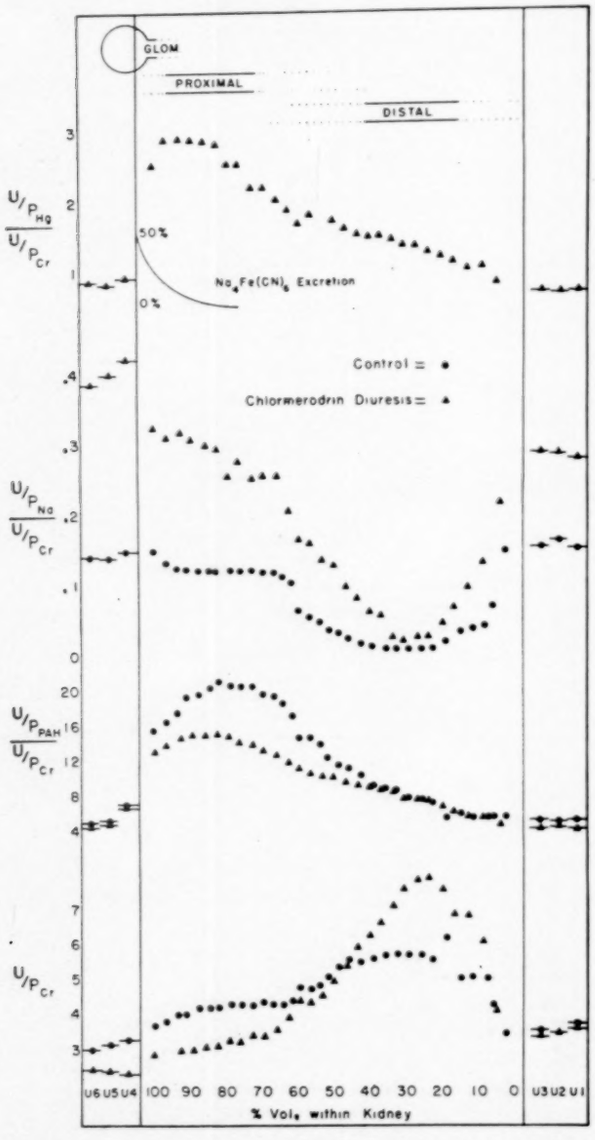


FIG. 6. Stop-flow localization of the diuretic action of chlormerodrin in the dog. (From KESSLER, R. H., *Am. J. Physiol.* [*73*].)

of the total tubular reabsorption of sodium and chloride ions [*65*]. The magnitude of the mercury-sensitive fraction is debatable but probably ranges between 15 and 30 per cent of the total. Does this mean that the sodium and chloride reabsorption of a specific portion of the tubule is blocked by mercurials, or does it mean that a specific fraction of sodium and chloride reabsorption distributed along the renal tubule is inhibited by mercury? One might paraphrase the second alternative in these terms: Is there one basic mechanism for sodium and chloride reabsorption and does it derive its energy from several types of enzymatic reaction, only one of which is sensitive to mercurials? It is unlikely that we will have a definitive answer to these questions until the sodium and chloride transport mechanisms are more precisely characterized.

One line of approach has been the study of pathological lesions in the renal tubules following the administration of inorganic or organic mercurial compounds. When minimal necrotizing doses are administered, the proximal tubule alone, and more specifically the distal portion of the proximal segment, shows evidence of pathological change [*19*]. However, the administration of large necrotizing doses of mercury damage all segments of the nephron. One must remember that even with the administration of minimal necrotizing doses one is dealing with alterations in cell structure which may be unrelated to the alterations in cell function induced by therapeutic doses.

One of the most characteristic reactions of inorganic salts of mercury and of those organic compounds with one free mercury valence is the formation of mercaptides with sulfhydryl groups. It is a reasonable although unproved inference to assign the diuretic properties of mercurials to their ability to inhibit sulfhydryl enzymes by forming inactive mercaptide complexes. Wachstein and Meisel, and others [*66,67*] have noted that the activity of succinoxidase, demonstrable histochemically by the neotetrazolium reaction, is inhibited by the prior administration to rats of mercurial diuretics in very large dosage (10 to 30 mg. Hg per kg. body weight). The inhibition is most pronounced in the third or "straight" position of the proximal segment. Unfortunately, doses equivalent to therapeutic doses in man have no demonstrable effect on succinoxidase activity of the kidney in rats. Therefore, inhibition of succinoxidase seems to have more pathological than functional significance. Cafruny et al. [*68*], using quantitative methods for histochemical identification of protein-bound sulfhydryl groups in sections of kidney, have observed a reduction in concentration in the proximal tubules, loops of Henle and collecting ducts following the administration of therapeutic doses of mercurials.

In man it is claimed that mercurial diuretics given in therapeutic doses reduce the reabsorptive Tm for glucose [*69*] and the secretory Tm for p-aminohippurate [*70,71*]. Since both functions are presumed to be localized in the proximal segment of the renal tubule, these findings have been advanced as evidence that mercurials block salt reabsorption in this same segment. Unfortunately for this thesis, neither glucose Tm [*72*] nor p-aminohippurate Tm [*71*] of the dog is reduced by mercurial diuretics.

It has been pointed out that mercurial diuretics do not interfere with acidification of the urine or with ammonia secretion [*19*]. Since both functions are presumed to be resident in the distal tubules, the inference has been drawn that mercurials could not exert their characteristic blockade of sodium reabsorption at this site. The dangers of such derived arguments are highlighted by the fact that mercurials do inhibit potassium secretion [*38*], a function no less sure in its distal localization than ammonia and acid secretion. Clearance observations have been variously interpreted as indicating proximal blockade and distal blockade of salt reabsorption, the validity of the evidence in either instance depending solely on the validity of the assumptions [*19*].

Recent studies of Kessler et al. [*73*] on the dog, utilizing the "stop-flow" technic of Malvin, Sullivan and Wilde [*45*] described earlier in connection with the action of acetazoleamide, have provided more objective evidence as to the site of action of mercurial diuretics. Figure 6 summarizes data obtained in two of their experiments, one a control (circles), the other following a dose of 1.0 mg. Hg per kg. body weight as chlormerodrin (neohydrin®). The chlormerodrin experiment was performed at the peak of the diuresis. The locus of maximum secretion of p-aminohippurate is considered to be proximal; the locus of maximum creatinine U/P ratio is considered to be distal. It is obvious that the major site of inhibition of sodium reabsorption is the proximal tubule. The activity of the distal mechanism, which can reduce urinary sodium essentially to zero, seems but little affected by the diuretic. It is interesting and significant that chlormerodrin is secreted by the proximal tubule ($U/P_{Hg}/U/P_{Cr} = 3.0$) and that maximal mercury secretion and maximal blockade of sodium reabsorption are coextensive within the nephron. It is possible that concentration of the diuretic in the proximal segment in the course of secretory transport of mercury is a factor of importance in inhibition of salt reabsorption in those same proximal tubular cells. Of some twelve organic compounds of mercury studied by Kessler et al. [*74*], all five which were diuretics were actively secreted by the renal tubules. Six of the seven which were devoid of diuretic properties were not secreted. One, p-chloromercuribenzoate, was not a diuretic, yet was actively secreted and reasonably well concentrated in the renal cortex in the process of secretory transport. This exception suggests caution in attaching too much significance to correlation of tubular transport and diuretic activity.

Nature of the Diuretic Process. It is a reasonable but unproved assumption that mercurial diuretics exert their characteristic actions by forming inactive mercaptide complexes with sulfhydryl enzymes vital to salt transport across the renal tubule. Such enzymes might be the direct carriers of ions or might be necessary for the provision of energy to the transport machinery. All diuretics have at least one potentially free valence of mercury, i.e., a structure $^{+}Hg—C—R$. Mercury compounds of the structure R—C—Hg—C—R have no diuretic properties. These latter compounds do not react with sulfhydryl enzymes; the former do. The affinity of the renal sulfhydryl enzymes for the free valence of mercury is greater than that of monothiols such as cysteine or glutathione but less than that of dithiols such as BAL. This statement derives from the fact that mercurials complexed with monothiols retain their diuretic properties [*75*]; those complexed with dithiols do not [*76*]. The following equation,

$$\text{Enzyme—SH} + {}^{+}\text{Hg—C—R} \rightleftharpoons \text{Enzyme—S—Hg—C—R}$$

implies that a reversible reaction between enzyme and diuretic results in an inactive complex. Dithiopropanol almost instantaneously blocks diuresis by complexing the mercurial and restoring the integrity of sulfhydryl groups.

$$\text{Enzyme—S—Hg—C—R} + \begin{array}{l}\text{HS—CH}_2\\ \quad\quad|\\ \text{HS—CH}\\ \quad\quad\|\\ \text{HO—CH}_2\end{array} \rightarrow \text{Enzyme—S—H} + \begin{array}{l}\text{R—C—Hg—SCH}_2\\ \quad\quad\quad\quad\text{HS—C—H}\\ \quad\quad\quad\quad\quad\quad\|\\ \quad\quad\quad\quad\text{HO—C—H}_2\end{array}$$

The diuretics in common use today are rapidly excreted in the urine, some 50 per cent of an intravenous dose being eliminated in two hours by the dog. Weiner and Müller [77] have shown that most of the diuretic is eliminated as the parent compound complexed with cysteine or

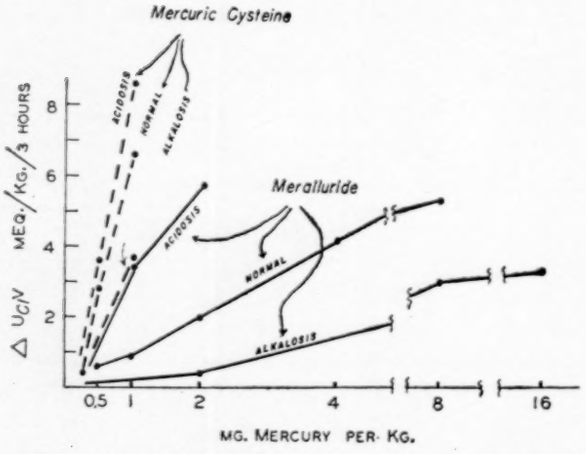


FIG. 7. Experiments illustrating marked potentiation of the diuretic action of meralluride by acidosis and little effect on that of mercuric cysteine. The data also illustrate the much greater diuretic activity of the ionizing compound, mercuric cysteine. (From LEVY, R. I., WEINER, I. M. and MUDGE, G. H. [84] and MUDGE, G. H. Mechanism of action of mercurial and xanthine diuretics. Proc. New York Acad. Sc. [22].)

acetylcysteine. Since the affinity of renal enzymes for the diuretic exceeds that of monothiols, some source of energy must be available to split the enzyme complex and secrete the diuretic.

$$\text{Enzyme—S—Hg—C—R} + \text{HS—CH}_2\text{CHNH}_2\text{COOH} \xrightleftharpoons{\text{Energy}} \text{Enzyme SH} + \text{R—C—Hg—S—CH}_2\text{—CHNH}_2\text{COOH}$$

Sollman et al. [78] some years ago postulated that organic compounds of mercury are diuretics by virtue of the fact that they decompose in the body to liberate mercuric ion. This view has been revived recently by Mudge and his colleagues [22,79] as a means of explaining the potentiation of the diuretic activity of organic mercurials by ammonium chloride. Mudge maintains that all organic mercurials which are diuretics are more or less labile and that lability is increased in acid solution. Inhibition of diuresis in metabolic alkalosis and enhancement of diuresis in metabolic acidosis is presumed to result from variable breakdown of a non-diuretic parent molecule into diuretic mercuric ion.

In view of Weiner and Müller's results [77], it is necessary to postulate that only a minute fraction of the large quantity of diuretic taken up by the kidney [80,81] and concentrated in the renal cortex [82,83] is concerned with diuresis. Mudge et al., in support of their thesis, have shown that

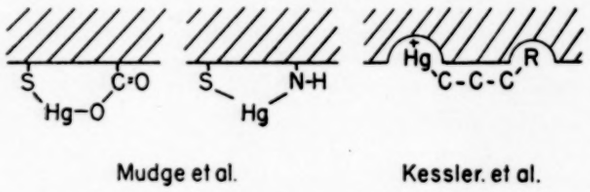


FIG. 8. Concepts of the mechanism of action of mercurial diuretics. On the left, a concept based on the view that inorganic mercuric ion is the diuretic substance. On the right, a concept based on the view that the parent molecule has diuretic properties.

the diuretic efficacy of meralluride is markedly affected by experimental alterations in acid-base balance whereas that of mercuric cysteine (a readily ionizable compound) is not. Figure 7 summarizes the views of Mudge et al. [84]. Diuretic efficacy is expressed on the ordinate as excess chloride excreted in a three hour period following an intravenous dose of the drug. The dose is plotted on the abscissa. They interpret these results as follows. Meralluride is relatively ineffective as a diuretic in metabolic alkalosis because it is stable in alkaline solution. Its activity is greatly enhanced in metabolic acidosis because of its acid lability. Mercuric cysteine is considerably more active under all conditions at far lower dose levels for all of it is potentially available as mercuric ion.

This attractive thesis cannot, however, be accepted in its entirety in the light of present knowledge. The infusion of acetazoleamide [62,85] or of potassium chloride [85], procedures which alkalinize the urine and presumably elevate the pH of tubular cells concerned with hydrogen ion exchange, does not alter the response to mersalyl and chlormerodrin. The induction of a marked extracellular and intracellular acidosis by the inhalation of 12 per cent carbon dioxide does not potentiate the action of mersalyl [62] or chlormerodrin [85]. Others have not found the response to inorganic mercuric ion to be so independent of acid-base changes as has Mudge. Finally, if inhibition of sulfhydryl enzymes is of significance in mercurial diuresis, it is not immediately apparent why compounds of the character $^{+}$Hg—C—R could not block them as well *in vivo* as *in vitro* [74]. According to

Mudge, divalent mercuric ion may be necessary to block two adjacent active sites, one a sulfhydryl group, the other an amino or carboxyl group. (Fig. 8.)

A somewhat different view has been outlined by Kessler, Lozano and Pitts [*74*] who suggested from their studies of a limited series of organic compounds of mercury that steric configuration of the parent molecule might be of significance. They proposed that for the diuretic to form a stable and inactive complex with the renal enzyme a terminal atom of mercury must be separated by three carbons or by an equivalent distance from a hydrophilic group. (Fig. 8.) Although they recognized certain exceptions which have subsequently been expanded by Mudge [*22*], they proposed this thesis as a working hypothesis. It is interesting that p-chloromercuri benzoate, methyl mercuric chloride and phenyl mercuric chloride, all potent inhibitors of sulfhydryl enzymes *in vitro*, are not diuretics. To avoid belaboring the point, it seems best to reserve for the future a decision as to whether the parent molecule is diuretically active or whether decomposition to mercuric ion is required.

Distribution and Excretion of Mercurial Diuretics. Mersalyl, meralluride and chlormerodrin disappear rapidly from the blood stream following intravenous injection [*80,86*]. Three major exponential rates determine the regression of plasma concentration: the most rapid one is dominated by mixing in the vascular compartment; the intermediate one, by diffusion into and binding by the tissues; and the slowest one, by renal excretion. The kidney, and more specifically the renal cortex, binds the mercurials more avidly than any other tissues [*82,83*]. BAL reduces renal binding and increases diffusion into other tissues [*80,82*]. Excretion of the organic mercurials is rapid in the dog [*74,80*] and in man and very slow in the rat [*82*]. Because of extensive plasma binding, little of the diuretic enters the glomerular filtrate [*74,80*]; essentially all of it is eliminated by tubular secretion, complexed with cysteine or acetyl cysteine. In comparison with the organic diuretics, mercuric chloride is excreted more slowly and the diuresis which it induces is more gradual in onset [*81*]. Otherwise its behavior in the body is much like that of the organic compounds.

A most interesting property of organic as well as inorganic compounds of mercury is their great affinity for and concentration in renal cortical tissue. They are highly concentrated in the kidney of the dog [*83*], rat [*82*], man [*87*], chicken, turtle, frog, necturus and fresh water fish [*88*] and in both glomerular and aglomerular marine teleosts [*89*]. Even the marine worm, Phascolosoma gouldi, concentrates them highly

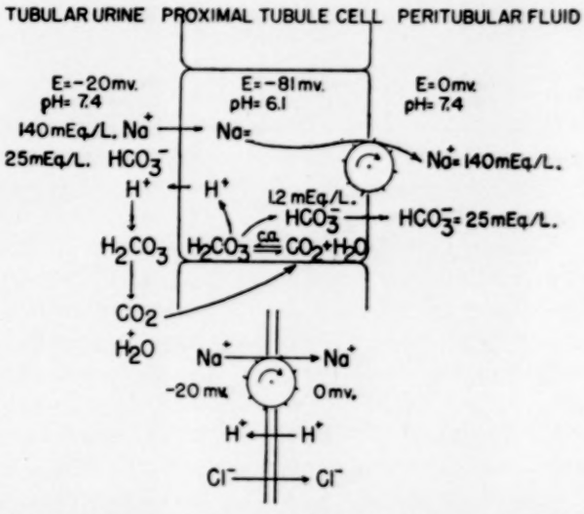


FIG. 9. Concept of a proximal tubular ion pump which would actively transport sodium ions and which would passively transport chloride ions. This is the same mechanism described in Figure 3. See text for details and assumptions.

in its excretory organ [*90*]. In the dog and rat homogenization and differential centrifugation have shown that mercurial diuretics are bound in highest concentration to the soluble proteins of the cells [*83*]. They are also firmly bound to the granular (mitochondrial) fraction of the cells. Why kidney cortex should differ so greatly from other tissues and whether the extremely high concentrations are, *per se*, related to and the cause of diuresis or whether they are more related to excretion are unknown. The problem is all the more confusing because certain organic mercurials are highly concentrated in the renal cortex, yet are slowly excreted and exhibit no diuretic activity [*74*].

Nature of the Proximal Tubular Mechanism for Reabsorption of Sodium and Chloride. If, as seems reasonable from the results of Kessler et al. [*73*], mercurial diuretics block a fraction of the proximal tubular reabsorption of sodium and chloride ions without appreciably affecting the distal process (except for specific depression of potassium exchange), we need consider only the nature of the former process in this discussion. The mechanism outlined in Figure 9 is merely an extension of that presented earlier in explana-

tion of proximal tubular reabsorption of bicarbonate. In fact one mechanism is responsible for the active reabsorption of sodium ions, for the passive reabsorption of chloride ions, and for the passive secretion of hydrogen ions (i.e., for bicarbonate reabsorption). The net influx of sodium ions will be exactly equal to the sum of the net influx of chloride ions plus the net outflux of hydrogen ions. This fact provides a basis for explanation of the mystifying (to me) reciprocal relationship between the thresholds for chloride and bicarbonate, but since it is not germane to our discussion, it will receive no further consideration here.

Sodium ions are actively pumped out of the cell and into the peritubular fluid, establishing the observed transcellular potential difference of 81 mv. and maintaining the intracellular sodium concentration at a relatively low value. Chloride diffuses out along the electrical gradient of 81 mv. to establish an equilibrium concentration ratio of $Cl_{(cell)}/Cl_{(peritubular\ fluid)} = 1/21$. If the peritubular concentration of chloride is 110 mEq./L., the intracellular concentration will be slightly over 5 mEq./L. Sodium diffuses passively into the cell from the tubular lumen down both electrical and chemical gradients. Chloride will also diffuse into the cell for the favorable chemical gradient into the cell exceeds the adverse electrical gradient. Thus the net electrical gradient of 61 mv. across the lumenal membrane is so oriented as to oppose the diffusion of chloride ions from lumen to cell. Were the cell concentration of chloride 11 mEq./L., no net diffusion of chloride would occur. With a cellular concentration of 5 mEq./L., passive inward diffusion is possible. A highly significant element of this scheme is the postulate that the permeability of the membrane to chloride is relatively less than that to sodium. In fact it is the difference in fluxes, based on differences in permeability, which establishes the lumenal potential of −20 mv., i.e., which reduces the potential difference between lumen and cell from 81 to 61 mv. It is this potential difference which accounts for the proximal secretion of hydrogen ions.

A more simple way to view the situation is to consider the tubular epithelium as a single membrane separating tubular lumen and peritubular fluid. The active transport of sodium establishes the observed potential difference of 20 mv. between lumen and peritubular fluid. The orientation of this potential is such as to favor passive diffusion of chloride ions from tubular lumen to peritubular fluid.

According to this view, mercurial diuretics interfere with the operation of the sodium pump. They do not stop it, rather they slow it down by 15 to 30 per cent, perhaps by cutting off one limited source of energy dependent on sulfhydryl enzymes. If this view is correct, one would predict that mercurials should reduce the transcellular and transtubular potential differences. Giebisch [*56*] has indeed observed that chlormerodrin reduces the transcellular potential in Necturus.

This discussion should not be regarded in any sense as proof of the validity of the concepts presented. It is merely aimed to show that, given a mechanism for the active transport of sodium in the proximal tubule, one can explain the reabsorption of chloride and bicarbonate as passive processes. That they are passive is not proved.

One cannot state that all actions of mercurial diuretics are exerted on the proximal segments of the renal tubules. The well documented inhibition of potassium secretion [*38*] must be exerted on the distal tubules. This does not necessarily mean that mercurials block directly the coupled lumenal transport mechanism which exchanges sodium ions for either potassium or hydrogen ions. (Fig. 4.) Were this mechanism directly blocked one would predict failure of acidification of the urine as well as inhibition of potassium secretion. It is possible that mercury acts one step back within the cell on some system which feeds potassium ions into the exchange mechanism, i.e., the analogue of the system which feeds hydrogen ions and which is blocked by carbonic anhydrase inhibitors.

CHLOROTHIAZIDE AS A DIURETIC AGENT

Chlorothiazide (diuril®), a heterocyclic sulfonamyl compound synthesized by Novello and Sprague [*91*], has recently been introduced as an orally effective diuretic agent. Because in some of its pharmacological properties it resembles acetazoleamide, in others, the mercurial diuretics, and especially because it sheds additional light on the mechanisms for reabsorption of sodium, we shall consider it briefly.

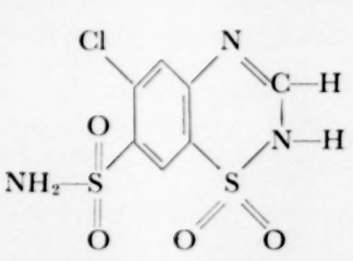
Chlorothiazide is an aromatic disulphonamyl compound in which one sulfonamyl group is cyclized with an acylamino group in a position ortho to it.

TABLE II*

AN EXPERIMENT ILLUSTRATING THE NATURE OF THE DIURETIC ACTION OF CHLOROTHIAZIDE IN THE DOG WHEN GIVEN IN MAXIMALLY EFFECTIVE DOSAGE

Urine Flow (cc./min.)	G.F.R. (cc./min.)	Plasma Clearance		pH		Excretion Rate (μEq./min.)				% of Filtered Reabsorbed			
		(mg. %)	(cc./min.)	Plasma	Urine	Na	K	Cl	HCO_3	Na	K	Cl	HCO_3
6.3	132	....	...	7.42	5.56	335	78	502	2	98	83	97	100
6.0	136	....	...	7.42	5.45	292	80	452	1	99	83	97	100
Chlorothiazide: 10 mg. per kg., prime; 15 mg. per kg. per hour, infusion													
11.2	127	3.08	267	7.44	7.27	488	223	1090	288	97	47	94	88
12.0	109	1.92	380	7.44	7.41	1380	287	1380	297	91	16	91	86
11.6	112	1.71	365	7.44	7.42	1350	233	1360	284	92	34	91	86
10.6	111	1.62	363	7.42	7.42	1350	202	1310	260	91	44	91	87
Chlorothiazide: 10 mg. per kg., prime; 30 mg. per kg. per hour, infusion													
10.2	108	5.38	256	7.43	7.40	1440	195	1380	273	91	41	91	86
9.8	107	3.60	341	7.43	7.37	1440	195	1340	275	90	42	91	85
9.0	112	3.22	349	7.42	7.43	1380	207	1310	265	91	40	92	86
8.0	115	3.10	341	7.42	7.37	1290	195	1220	244	92	41	92	87

* From PITTS, R. F., KRÜCK, F., LOZANO, R., TAYLOR, D. W., HEIDENREICH, O. P. A. and KESSLER, R. H. Studies on the mechanism of diuretic action of chlorothiazide. *J. Pharmacol. & Exper. Therap.* (in press) [*94*].



Beyer [*92,93*] has shown that chlorothiazide exhibits properties both of carbonic anhydrase inhibitors and of mercurial diuretics. Due to the fact that the molecule contains a free sulfonamyl group, it inhibits carbonic anhydrase *in vitro* and in relatively large doses *in vivo* alkalinizes the urine and increases the urinary excretion of potassium. In addition it causes natriuresis and chloruresis, actions similar to those of mercurial diuretics. In fact in the usual orally effective doses the major response is chloruresis and natriuresis. The urine may fail to become alkaline, although potassium excretion is usually enhanced.

Pitts et al. [*94*] administered chlorothiazide to dogs in doses sufficient to produce a maximal renal response. At the peak of diuretic action of the drug, either acetazoleamide or chlormerodrin was given in a dose sufficient to produce its maximal renal response. The order of drug administration was then reversed. Table II summarizes an experiment with chlorothiazide illustrating not only the basic elements of its diuretic action but also the fact that in the dose administered peak effects were obtained. It is apparent that a priming dose of 10 mg. per kg. and a rate of infusion of 15 mg. per kg. per hour induced natriuresis, kaluresis, chloruresis and bicarbonate excretion which were not significantly increased by repeating the prime and by doubling the rate of infusion. That peak action was attained from the lower dose is apparent on examination not only of the absolute rates of ion excretion but also of percentages of the filtered ions which are reabsorbed. In other experiments it was shown that a priming dose of 2.0 mg. Hg per kg. as chlormerodrin and a rate of infusion of 2.0 mg. Hg per kg. per hour produced a maximal renal response not increased by repeating the prime and doubling the rate of infusion. In Table III is summarized an experiment in which a dose of chlormerodrin sufficient to

TABLE III

AN EXPERIMENT ILLUSTRATING THE SUMMATION OF THE DIURETIC ACTIVITIES OF CHLORMERODRIN AND CHLOROTHIAZIDE IN THE DOG WHEN BOTH DRUGS ARE GIVEN IN MAXIMALLY EFFECTIVE DOSAGE

Urine Flow (cc./min.)	G.F.R. (cc./min.)	Chlormerodrin		Chlorothiazide		pH		Excretion Rate (μEq./min.)				% of Filtered Reabsorbed			
		Plasma (μg./cc.)	Clearance (cc./min.)	Plasma (mg. %)	Clearance (cc. min.)	Plasma	Urine	Na	K	Cl	HCO_3	Na	K	Cl	HCO_3
6.7	52.2	....	....	....	...	7.31	6.26	390	21	440	9	95	88	94	99
7.2	51.2	....	....	....	...	7.31	6.35	420	37	480	13	95	80	93	99
Chlormerodrin: 2 mg. Hg per kg. prime; 2 mg. Hg per kg. per hour, infusion															
7.3	48.6	37.2	4.1	....	...	7.32	6.35	460	35	690	12	94	80	90	99
10.3	49.4	22.0	12.2	....	...	7.32	6.01	880	35	960	8	88	80	86	99
14.2	49.4	20.6	12.1	....	...	7.32	5.88	1520	41	1650	7	79	77	76	99
15.7	46.1	19.5	13.6	....	...	7.32	5.79	1850	41	1980	6	73	76	70	99
14.8	41.1	20.8	11.8	....	...	7.32	5.67	1830	42	1950	4	70	74	67	99
Chlorothiazide: 10 mg. per kg., prime; 15 mg. per kg. per hour, infusion															
16.9	37.7	19.8	13.7	5.10	87	7.34	6.86	2280	46	2330	97	59	68	56	84
15.8	35.7	17.8	16.2	2.55	163	7.34	6.86	2200	41	2230	92	59	70	55	83
13.0	30.8	17.8	16.4	2.23	132	7.36	6.61	1790	38	1880	42	61	67	56	91
12.8	31.4	14.7	18.4	2.17	130	7.39	6.49	1770	48	1880	28	62	59	57	94

* From PITTS, R. F., KRÜCK, F., LOZANO, R., TAYLOR, D. W., HEIDENREICH, O. P. A. and KESSLER, R. H. Studies on the mechanism of diuretic action of chlorothiazide. *J. Pharmacol. & Exper. Therap.* (in press) [91].

produce its maximal renal response was administered first, followed by a dose of chlorothiazide sufficient to produce its maximal renal response. It is apparent that the natriuretic and chloruretic actions of chlormerodrin and chlorothiazide are additive when each is given in maximal dosage. From this it was concluded that the two drugs block two different reabsorptive mechanisms or the same mechanism in two distinctly different ways which sum more or less perfectly. Since large doses of chlormerodrin and chlorothiazide administered together block somewhat less than half the total reabsorption of sodium and chloride, there must be some sort of third mechanism. We are inclined to the view that there is but a single mechanism of sodium reabsorption with multiple sources of energy. Chlormerodrin blocks one source of energy; chlorothiazide blocks another. A third, a fourth or more energy yielding sources are resistant to the inhibiting actions of both drugs.

Experiments combining chlorothiazide and acetazoleamide led to the conclusion that both drugs, with respect to their carbonic anhydrase inhibiting actions, block common mechanisms of bicarbonate reabsorption and acidification and facilitate a common mechanism of potassium secretion. In these actions, acetazoleamide is the more potent of the two drugs.

CONCLUDING REMARKS

Gilman [21], in a recent review of the diuretic activities of carbonic anhydrase inhibitors, has pointed out their great heuristic value with respect to renal transport mechanisms. His statement applies equally to mercurial diuretics, to osmotic diuretics and to a lesser extent to xanthine diuretics, the antialdosterones and the new antisecretory agents. The reverse side of the coin bears some scrutiny too. As more is learned about renal transport mechanisms, that knowledge can be channeled into improved treatment of patients exhibiting disturbances of acid-base and salt and water balance and into the development of better therapeutic agents.

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The Kidney in Potassium Depletion*

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DURING the past twenty years an increasing body of experimental and clinical evidence has lent support to the proposition that a deficiency of potassium may produce significant alterations in renal structure and function [*1–4*]. In the renal tubular cells, potassium plays a dual role. As in all other cells, it is probably an essential activator of some of the enzymatic reactions maintaining the integrity of the tubular cell and supplying the energy required for its specialized activities. Beyond this, however, potassium is itself one of the ions transported across the renal epithelium as part of the interrelated series of ionic exchanges by which the kidney contributes to the maintenance of body electrolyte and acid-base equilibrium. These two roles undoubtedly are intimately related. Nevertheless, in our present state of knowledge the various renal abnormalities resulting from potassium depletion seem to be conveniently discussed in terms of one or the other of these functions. This point of view will provide the framework for the present summary of the effects of potassium depletion on the kidney.

Renal Pathology in Experimenal Potassium Depletion. Dietary deficiency of potassium in rats and mice produces enlargement of the kidneys and striking changes in their tubular structure [*5–9*]. The increase in renal mass begins within a few days and progresses for ten days to two weeks, the final weight often being two or more times normal [*10,11*]. The enlargement is absolute as well as in relation to body weight and is due to a nearly proportional increase in the content of solids and water [*8–13*], as well as of fat and glycogen [*10*]. Microscopic changes are first noted one week after starting the potassium-deficient diet and are well advanced by two weeks [*8,11,14*]. The most striking changes are found in the collecting tubules where there is swelling, hyperplasia and prominent cytoplasmic granulation of epithelial cells [*7,11,14,15*], as well as enlargement of nuclei [*16*]. In addition, many collecting tubules show marked dilatation [*7,11,14,15*]. By means of microdissection of nephrons, Oliver and his associates [*14*] have demonstrated that these lesions occur in separate portions of the collecting system. The granular lesion is found in the innermost zone of the medulla, which is otherwise entirely normal, and extends out to the epithelium of the papillae. Swelling and hyperplasia of the clear cells, and a distinctive proliferation of the so-called intercalated cells, are confined to the tubules of the inner stripe of the outer zone of the medulla. Basement membranes in this area are notably thickened. Dilatation is found proximal to these lesions, in the proximal portions of the collecting system and occasionally in the distal convoluted tubules. For this reason it is suggested that dilatation is a passive consequence of the proliferative cellular occlusion situated distally [*14*]. These workers further noted that, except for minor patchy degenerative changes in the mid-portion of the proximal convolution, the remainder of the nephron is essentially normal. Although the earliest and most consistent lesions are found in the distal tubular system, many observers [*7,8,11,15–17*] have reported a variety of changes in other portions of the nephron. There is considerable diversity in the description of the nature and location of these lesions. Hydropic swelling, fatty degeneration, alterations in nuclear and mitochondrial staining, necrosis and even calcification have been reported. Some of the discrepancy in the descriptions of the more proximal lesions may be related to the severity or duration of the potassium depletion since the

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cortical changes appear to follow those in the medulla [*14*].

Restoration of potassium to the diet has been reported to result in virtually complete healing of the characteristic lesion [*17*].* It has also been noted that, several weeks or months after correction of a deficiency which had lasted six to eight weeks, rat and mouse kidneys showed interstitial scarring, hyalinized glomeruli and dilated tubules [*7,19*]. Thus, while the lesions of experimental potassium depletion are potentially reversible, at least at some stage, it appears that a point may be reached at which irreversible damage may occur, with resultant extensive scarring and atrophy of the renal substance.

Potassium depletion also produces changes in enzyme activity, as determined by histochemical methods. These are found mainly in the areas which appear to be most affected in ordinary histologic sections. There is increased acid phosphatase [*15,21*] and TPN diaphorase activity [*21*] in the collecting tubules, and a decrease in DPN diaphorase activity [*21*]. It has also been reported that non-specific esterase activity is markedly increased in both the collecting and the proximal tubules [*21*]. Variable and small changes in alkaline phosphatase have been noted [*11,21*], but succinic dehydrogenase, 5-nucleotidase and phosphorylase are unaffected [*15,21*]. The functional significance of these changes remains to be determined.

Tissue Composition in Experimental Potassium Depletion. The potassium content of renal tissue is usually reduced in experimental potassium depletion but the change is less than that found in muscle. With but one exception [*13*], all studies of kidney electrolyte content in rats have shown a significant 10 to 20 per cent reduction in potassium [*6,10,22,23*] which was roughly proportional to, and about 40 per cent of the magnitude, of the muscle potassium depletion. Of interest is the observation that, in contrast to muscle tissue which gains sodium as it loses potassium, no significant change in the sodium content of renal tissue could be demonstrated in deficient rats. In potassium deficient dogs, despite a 30 per cent reduction in muscle potassium, no changes in kidney electrolyte content were observed [*24*].

* It is of interest that addition to a potassium-free diet of rubidium or cesium (alkali metals closely related to potassium) prevents the appearance of renal lesions [*18*]. Furthermore, rat kidneys with extensive potassium deficient lesions can be restored rapidly to normal by treatment with rubidium chloride [*20*].

In the muscle of depleted rats some potassium is apparently replaced by the cationic amino acids, lysine and arginine [*25*], and analyses of kidney tissue also disclose a significant increase in these substances [*26*]. Of further interest in this connection is the observation that the muscle content of aspartic acid and glutamic acid falls, but that of kidney does not [*26*]. Since glutamic and aspartic are the only two amino acids with net negative charges at the pH of body fluids, it is possible that they act as expendable anions which leave the cell with potassium when there is marked loss of potassium, as in muscle, but not with the milder degrees of depletion which occur in the kidney.

Assays for carbonic anhydrase and glutaminase in the kidneys of potassium-depleted rats indicate an increase in the activity of both enzymes [*12*]. In the potassium-depleted dog, despite the absence of detectable change in tissue potassium content, the activity of these enzymes was also found to be increased [*24*]. However, if alkali is added to the diet of potassium-deficient rats or dogs glutaminase activity will be reduced rather than increased [*24*].

The Renal Pathology of Potassium Depletion in Man. It is now well recognized that renal lesions may accompany potassium deficiency in man [*2–4*]. The most striking finding is a vacuolization of the tubular epithelial cells. These vacuoles usually do not stain for fat, glycogen or protein, and these characteristics, taken with their large size and great number, serve to distinguish them from other degenerative epithelial lesions. It was first clearly recognized by Perkins, Petersen and Riley [*27*] and by Luft, Ringertz and Sjogren [*28*] that this tubular change was associated with potassium deficiency in man. In retrospect, however, numerous good descriptions of the same lesion can be found in earlier publications [*29–36*]. In many cases data on potassium were not available, but the clinical circumstances were such (e.g., chronic diarrhea) as to suggest the strong likelihood of severe potassium depletion. A particularly striking example of vacuolar nephropathy in a patient with diarrhea and known deficiency of potassium was reported by Williams and MacMahon [*31*].

In the past few years, since the recognition of the lesion, many descriptions of potassium depletion nephropathy have appeared [*2,37–53*]. Table 1 lists the diseases in which the renal tubular lesions now known to be characteristic of potassium depletion have been described.

These disorders, although of diverse etiology, have in common a trend toward severe chronic depletion of potassium. Diarrheal diseases seem to be the most common offenders but potassium-depletion lesions have also been noted in patients with hyperadrenalism [*45–54*], in those with renal tubular defects leading to potassium-wasting [*32,33,41,44*], in patients with other chronic renal diseases who become potassium depleted from extrarenal losses [*20,40*], and in those with ureterosigmoidostomy [*43*].

TABLE 1
CLINICAL DISORDERS ASSOCIATED WITH TUBULAR LESIONS OF POTASSIUM DEPLETION

Potassium depletion from gastrointestinal losses:
Ulcerative colitis
Regional enteritis
Cholera
Bacillary dysentery
Sprue syndrome
Whipple's disease
Malabsorption syndrome
Overuse of laxatives
Ureterosigmoidostomy
Hyperadrenalism:
Cushing's disease
Primary aldosteronism
Cortisone treatment
Renal disease with urinary potassium wasting:
Fanconi syndrome
Renal tubular acidosis
Cystinosis
Renal disease with extrarenal potassium wasting:
Subacute glomerulonephritis
Acute tubular necrosis
Chronic pyelonephritis

While the vacuoles in the convoluted tubular epithelium may be the most easily recognized of the renal changes produced by potassium depletion, there is good reason to believe that other less specific, and sometimes less striking, alterations in the tubular epithelium may occur. Granularity or foamy swelling of cytoplasm, necrosis and sloughing of cells, dilatation of tubules with atrophy of the epithelium, and even calcification of cells have been noted in one or more reports [*2,28,33,35,36,38,44,49*]. These changes are sometimes found together with the vacuoles, but in other cases no discrete vacuoles can be found.

The tubular lesions seem to be confined chiefly to the convoluted tubules, the collecting tubules in most cases appearing normal. Glomeruli also have been usually described as normal, although Jensen, Baggenstoss and Bargen [*35*] have called attention to the endothelial proliferation in some of their patients with ulcerative colitis. In patients with aldosteronism, hyalinization of glomeruli and areas of interstitial scarring and tubular atrophy can probably be attributed to the hypertensive disease and pyelonephritis which may complicate this condition [*38,50,52*]. With these exceptions, most patients with potassium-depletion nephropathy have normal renal blood vessels and interstitial tissue. Finally, it is of interest that a slight increase in renal weight (combined weight above 330 gm.) has been noted in a few instances [*27,28,35,36*].

In summary, then, the renal lesion of potassium deficiency in man resembles that produced experimentally in the rat in being confined chiefly to the tubular epithelium. In man the change is primarily vacuolar and degenerative and limited to the convoluted tubules. However, in rats there is a hyperplastic obstructing lesion in the collecting system which leads only secondarily to proximal changes, which include dilatation and some degeneration and necrosis. In both species renal hypertrophy may occur, and in neither are there significant lesions of glomeruli or blood vessels.

Virtually no precise information is available concerning the minimum duration and severity of the potassium deficiency required to produce significant lesions in man. By history, most of the patients observed to date appear to have been losing potassium for at least a month, and usually for many months or several years. Typical lesions have been noted within seven days after ureterosigmoidostomy [*43*], suggesting that they may develop much more rapidly in certain cases. At some stage of its development the tubular lesion in man is reversible, as demonstrated by serial biopsy study in two patients [*2*], but there are no data concerning the speed and frequency with which such healing might be expected to occur. The question of possible irreversible lesions, with scarring, atrophy and loss of nephrons, is not easily resolved because of the fact that patients with potassium depletion may suffer from other renal diseases which destroy kidney tissue.

It has been suggested that there is a high incidence of pyelonephritis in potassium-depletion nephropathy as a consequence of the increased susceptibility of renal tissue already damaged by potassium deficiency [*4,55*]. A review of the pathological description in thirteen reported cases of nephropathy caused by diarrhea [*2,27,28,31,34,37,39,40,42*] reveals that pyelone-

phritis was described in only two. Kulka, Pearson and Robbins did not mention pyelonephritic lesions in any of their fifteen patients [*36*] and Jensen, Baggenstoss and Bargen [*35*] noted the occurrence of pyelonephritis in only four of nineteen patients. In cases of aldosteronism there may be a somewhat higher incidence of pyelonephritis. In two of the published cases of aldosteronism [*52,56*] the patients were found to have pyelonephritis on renal biopsy and in two others positive urine cultures were obtained [*50,57*]. Two of three patients seen by the present authors [*20*] also have had pyelonephritic scars. On the other hand, in six other cases no histological or clinical evidence of pyelonephritis was noted [*47–49,51,53,58*].

The evidence for a predisposition to pyelonephritis is therefore unconvincing in the nephropathy associated with diarrhea, but somewhat more suggestive in aldosteronism. Cases of potassium-depletion nephropathy and pyelonephritis associated with renal tubular diseases, such as renal tubular acidosis and Fanconi's syndrome, and in patients with ureterosigmoidostomy, have been excluded from consideration in this connection since pre-existing infection, obstruction, stone formation and other metabolic and structural abnormalities in the kidneys make interpretation impossible. Finally, the possibility must be considered that potassium depletion itself might cause interstitial scarring which could mimic the lesion of chronic pyelonephritis. The recent observations [*19*] in chronically depleted rats would lend this suggestion some support, but this entire question needs much further investigation.

Renal Function in Experimental Potassium Depletion. In view of the histological changes produced in the kidney tubules it is not surprising that experimental potassium depletion is also associated with disturbances in renal tubular function. The most prominent abnormality is a defect in renal concentrating power, the severity of which appears to be proportional to the degree of tissue potassium depletion [*59*]. In rats in which muscle potassium was reduced by half as the result of two and a half weeks of a potassium-free, alkaline diet, the maximum urine osmolal concentration achievable with administration of vasopressin was reduced from the normal value of approximately 2,600 mOsm./K. to 1,000 mOsm./K. [*59*]. It has been suggested that final concentration of the urine occurs in the collecting tubules [*60,61*] and it is therefore of considerable interest that the initial and most striking tubular lesions in potassium-depleted rats are found at this site.

Polydipsia and polyuria appear as soon as rats [*10,11,62*] or dogs [*62,63*] are started on a low potassium diet. Initially, at least in the rat, these symptoms cannot be attributed to the concentrating defect because they precede the latter and occur prior to the development of significant potassium depletion [*59*]. A similar diabetes insipidus-like syndrome has been produced in dogs given DOCA [*64–66*]. The DOCA syndrome resembles that which results from a low potassium diet in that the polyuria and polydipsia can occur without the development of potassium deficiency [*66*].

The exact mechanism by which, in the absence of potassium deficiency, DOCA and a diet deficient in potassium promptly produce polyuria is not clear at present. While it has been suggested that polydipsia rather than a renal abnormality may be at fault [*65*], the available data do not adequately settle this point. The possible role of changes in glomerular filtration rate remains to be determined.

Although glomerular lesions are not a prominent feature of potassium-depletion nephropathy in animals, there are some suggestions that glomerular function may be impaired. Endogenous and exogenous creatinine clearances were found slighty reduced in rats depleted of potassium [*67*]. However, since protein depletion usually accompanies severe potassium depletion, and in view of the fact that protein depletion alone was found to reduce creatinine clearance, it was not possible definitely to attribute the changes to potassium depletion. Other studies have noted that azotemia may develop in potassium-depleted rats [*11,19*] and sometimes may persist long after correction of the deficit [*19*]. Further information is obviously required to clarify the significance of these observations.

Renal Function in Potassium-Depleted Man. In man, the renal lesion of potassium depletion characteristically produces a defect in the tubular reabsorption of water. Almost all patients studied have had isosthenuria, resistant to vasopressin. Thus, for example, nineteen of twenty-one patients with primary aldosteronism [*20,46–53,56–58,68–72*] could not produce a concentrated urine, and this defect was noted in all five cases of potassium-depletion nephropathy due to diarrhea recently studied by the present authors [*2*] as well as the six other similar cases collected from

the literature [2]. In some instances this defect has been associated with marked polyuria and polydipsia. Normal subjects maintained on kaliuretic adrenal steroids develop polyuria, nocturia, and vasopressin-resistant isosthenuria after several weeks [20].

In view of current suggestions that final concentration of the urine may occur in the collecting tubules [60,61], the absence of significant lesions in this area in the human material is noteworthy. It should be recalled that the concentrating defect in rats is associated with a striking lesion of the collecting tubules. This difference may well be due to an histologically inapparent but functionally important lesion in the human-collecting tubule. On the other hand, it is possible that in man final concentration occurs proximal to the collecting tubules, or that potassium depletion impairs a step in the urine concentrating mechanism which precedes the final distal reabsorption of water.

Other tubular functions have not been systematically studied in potassium-depleted man. However, in several patients with depletion caused by diarrhea, excretion of phenol red was reduced, and then rose as potassium balance was restored [2]. Similar changes have not been found in patients with primary aldosteronism. Clearance of PAH was reduced in a number of patients with both types of potassium depletion [2,48,56,57,69,73]. The disproportionately low levels of PAH clearance, as well as measurements of TmPAH and renal extraction of PAH in one patient [2], would suggest that at least some of this reduction in clearance is due to impairment of tubular extraction rather than entirely to diminished effective plasma flow. The importance of potassium in PAH transport is indicated by the observation that the accumulation of PAH by rabbit kidney slices is maximal only when tissue potassium is normal [74].

Blood nitrogen levels have as a rule been within normal limits in the patients with aldosteronism, but in a few with diarrhea mild elevations have been noted [2,31]. Glomerular filtration rate, as measured by inulin clearance, or estimated by endogenous creatinine clearance, has been found usually moderately depressed in patients depleted by diarrhea [2]. In some cases these changes were reversed by restoration of potassium stores. In those with aldosteronism, the filtration rate has usually been normal, but in a few instances moderate reductions have been noted [20,48,52,56,57]. Nephrosclerosis and pyelonephritis complicated most of the latter cases, however, and interpretation of these changes is difficult. It is of interest, nevertheless, that in eight patients with aldosteronism in whom a tubular concentrating defect was marked, the glomerular filtration rate appeared to be within normal limits [20,46,49,50,53,69].

In many instances the functional defects have proved to be largely or entirely reversible following correction of the potassium deficit. In nine of sixteen cases of aldosteronism in which data are available, operative cure of the condition was followed by either marked improvement in concentrating ability or restoration of this function to normal [20,46,50,68,69,72]. In the other seven cases there was either no change in concentrating ability or very slight improvement [48,49,52,53,56,69]. A few cases improved within three or four weeks of operation. The patient reported on by Eales and Linder [50] showed slight improvement three weeks postoperatively, and had regained normal concentrating ability twelve weeks postoperatively. With this exception, there are no serial studies adequate to define the time course of the improvement of function after cure of aldosteronism. A few serial measurements are available in three patients recovering from diarrhea-induced depletion [1,2]. Immediately following repletion all functions had improved, but were still below normal. One to three months later concentrating ability as well as inulin, creatinine and PAH clearances were restored to normal limits.

To summarize: The available data suggest that in man, as in experimental animals, the most consistent functional defect associated with potassium-depletion nephropathy is to be found in the tubules, with pitressin-resistant isosthenuria as the chief characteristic. Other tubular disturbances may occur, but there are insufficient data on this point. The glomerular filtration rate is often reduced in diarrhea-induced deficiency, but usually is within normal limits in aldosteronism. Gradual restoration of all deranged functions may occur after repair of the potassium deficit, but the question of permanent irreversible defects has not yet been settled.

Potassium Deficiency and The Renal Regulation of Electrolyte and Acid-Base Equilibrium. The discussion has thus far been concerned with those structural and functional consequences of potassium depletion which can probably be explained by the role of potassium as an essential com-

ponent of the general metabolic processes of tubular cells. However, as an integral part of the complex ion transport mechanisms by which renal regulation of electrolyte and acid-base balance is accomplished, the potassium ion has still another role to play. The following section deals with this latter aspect of potassium depletion, namely its effect on the renal tubular handling of potassium and related electrolytes.

A. *Excretion of Sodium and Potassium.* The normal kidney is so efficient in its conservation of sodium that excretion of this ion is reduced to only a few milliequivalents a day within five days after dietary sodium is abruptly withdrawn [*75*]. Conservation of potassium after withdrawal is much less effective. Only after two weeks on a potassium intake of 10 to 15 mEq. does urine potassium approach this figure, at a time when the total potassium deficit is perhaps 250 to 300 mEq. [*76–78*]. Restriction of sodium intake has been reported to increase the efficiency of potassium conservation [*79*]. Ultimately, in normal experimental subjects [*80*] and in patients with more severe potassium deficiency [*1,2,81,82*] the urinary excretion of potassium drops off to only a few milliequivalents per day. The capacity for renal conservation of potassium is further demonstrated in deficient subjects by a considerable reduction in the acute response to such kaliuretic stimuli as hyperventilation, osmotic diuresis and the administration of sodium bicarbonate, acetazoleamide, potassium chloride or ammonium chloride [*83,84*]. It has also been shown that the normal daytime diuresis of potassium disappears, with the result that excretion tends to remain constant throughout the twenty-four hour cycle [*83*]. Even in cases in which tubular function has been significantly impaired by potassium-depletion nephropathy, renal conservation of potassium may nevertheless be very efficient [*1,2*]. In this respect patients who become depleted as the result of hyperadrenalism or primary renal tubular defects differ from those whose deficits derive from low intake, vomiting or diarrhea. In the former group, urine potassium remains normal or increased, despite falling plasma levels and body stores. Thus a very low excretion of potassium ($<$10 mEq. per day) usually indicates severe and fairly long-standing potassium depletion, and virtually excludes the possibility of hyperadrenalism or a primary potassium-wasting tubular defect.

The renal handling of sodium appears to be affected by potassium depletion. Sodium and chloride retention are known to occur during the development of experimental potassium deficiency [*76–78,85*]. The converse relationship has also been observed, namely that administration of potassium to depleted subjects immediately increases sodium excretion [*76–78, 86*]. Excretion of sodium loads is delayed in patients with potassium deficiency due to diarrhea or low intake, and these patients tend to become edematous when given sodium [*1,86*]. This phenomenon may also be responsible for the retention of sodium and the overexpansion of extracellular space which follows treatment of patients depleted of potassium by diabetic acidosis [*87*] and severe diarrhea [*88*].

The mechanism relating potassium depletion to sodium excretion is not entirely clear. Retention of sodium may be due in part to reduced glomerular filtration in patients with diarrhea [*1*], but Fourman found no changes in endogenous creatinine clearance in one experimental subject in whom edema with sodium loading developed [*86*]. There is no evidence to suggest an important hormonal factor, since aldosterone production tends to fall with potassium depletion [*89*], and aldosterone excretion has been found to be decreased in a patient with laxative-induced depletion who had marked edema [*90*]. A possible explanation of the sodium retention is suggested by Berliner's hypothesis that a sodium-hydrogen exchange is responsible for the reabsorption of sodium chloride as well as the excretion of acid [*91–93*]. He has further suggested that depletion of cellular potassium in the renal tubules tends to favor the secretion of hydrogen [*91–93*]. If this were so, potassium depletion would be expected to accelerate sodium chloride reabsorption, and loading with potassium to increase sodium chloride excretion. In potassium depletion associated with aldosteronism, the excretion of sodium loads is rapid [*20*] and edema does not usually occur [*94*]. This may be due to the higher filtration rate in this condition as compared to other types of potassium deficiency, but the question cannot be definitely answered on the basis of present information.

B. *Regulation of Acid-Base Equilibrium.* The renal threshold for bicarbonate is increased in potassium deficiency [*91,92,95,96*] as, in fact, must always be the case when there is protracted elevation of the plasma bicarbonate concentration [*97*]. There is good evidence to suggest that

bicarbonate reabsorption is accomplished by a sodium-hydrogen exchange in the tubule [*98*], and a reduction in renal tubular potassium concentration is thought to accelerate this process [*91,92*]. According to this hypothesis, hydrogen and potassium compete for a common secretory pathway, and a decrease in potassium tends to favor hydrogen secretion. It has also been shown, however, that bicarbonate reabsorption is increased by an elevated blood pCO_2 [*97,99,100*]. Therefore, to the extent that pCO_2 rises with respiratory compensation in chronic alkalosis, an increased renal threshold for bicarbonate might be expected [*97*]. Since there is evidence to indicate that tissue pH is decreased in potassium depletion [*101,102*] as well as in hypercapneic states, it has been suggested that the effect of both conditions on the bicarbonate threshold may be mediated through a change in cellular pH [*96,103*].

Whatever the mechanism, it is clear that in the alkalosis of potassium depletion the urine contains less bicarbonate than would normally be expected at similar plasma bicarbonate levels, and in this sense there is "paradoxical aciduria." Actually, however, the urine pH need not be very low for the bicarbonate threshold to be increased [*97*]. During the development of experimental potassium depletion, in fact, the pH of the daily urine collections tends to rise [*77,83,84*] and the normal nocturnal aciduria disappears [*83*]. In primary aldosteronism all urine specimens are relatively alkaline, the pH rapidly becoming more acid when the potassium deficit is repaired [*47,48,70,104*].

Despite the tendency toward a high urinary pH in potassium depletion, there is no reduction in excretion of ammonium. The urine excretion of this ion in depleted humans is higher than would be anticipated from the urine pH [*1,84*] and administration of potassium rapidly returns it to more normal levels. Increased urine ammonium excretion has also been noted in experimentally depleted rats [*12*] and dogs [*24*]. The increase in renal glutaminase, which has already been mentioned as a consequence of experimental potassium depletion [*12,24*], may be one cause of the increased ammonia, but other factors such as the reduction in tubular cell pH and potassium concentration, and the tendency for increased sodium reabsorption [*76–78,85*] may play important roles. In patients with aldosteronism the relatively high urinary ammonium may be due in part to the direct tubular action of the aldosterone, since it has been shown that administration of sodium-active steroids to normal subjects causes an immediate rise in ammonium excretion [*105*], presumably by increasing the reabsorption of sodium at the site at which ammonia enters the filtrate. In normal subjects given steroids [*105*] and in those with aldosteronism [*20*], the ammonium excretion is reduced by a low sodium intake. Sodium loading of potassium-deficient animals, furthermore, will accelerate the already increased ammonium excretion [*12*]. These observations lend more support to the suggestion that increased reabsorption of sodium in some way activates ammonium excretion.

Potassium depletion thus appears to produce paradoxical effects on the renal tubule. On the one hand there is an increased threshold for bicarbonate and a tendency to increased excretion of ammonium, both of which suggest an acceleration of hydrogen secretion. On the other hand, there is a tendency for the urine pH to be persistently alkaline with the consequent loss of modest amounts of bicarbonate in the urine, which would seem to indicate a defect in hydrogen ion secretion. It should be noted that the net result of these defects tends to stabilize the blood bicarbonate since the simultaneous increases in ammonium and bicarbonate excretion tend to cancel each other. It cannot be stated at present whether the bicarbonate "leakage" represents a primary defect in the establishment of hydrogen ion gradients distally in the potassium-depleted tubule, whether it is simply a passive consequence of the initial increase in ammonium excretion, or whether these represent two independent but complementary changes in tubular function, one in the proximal tubule and the other located more distally.

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Mechanisms of Renal Tubular Transport*

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OUR present concepts of renal tubular function are surprisingly recent in their origins. The eighty years (1842–1922) that followed the publication of Bowman's classical monograph on the structure of the glomerulus were more productive of controversy than of new information which could be considered helpful in appraising the relative contributions of glomerular filtration, tubular reabsorption and tubular secretion in the elaboration of urine. Throughout this period, two principal schools of thought contended for supremacy. One, representing the views of Bowman, Heidenhain and their adherents, held that tubular secretory activity accounted for the addition of the urinary solutes to water separated from the blood by glomerular filtration, the water serving only to sweep out the secretory products. Ludwig and his followers, in contrast, proposed the formation of a protein-free ultrafiltrate of plasma at the glomeruli, with subsequent concentration of the urinary solutes by water reabsorption in the tubules. It is noteworthy that as late as 1917, Cushny's "modern theory" of kidney function envisioned little more than the ultrafiltration of plasma and the tubular reabsorption of an ideal solution approximating Locke's solution in composition.

The application of micropuncture technics in 1923 by Richards, Walker and their colleagues [*1,2*] permitted the sampling and analysis of capsular and tubular fluids and provided the first unequivocal evidence for glomerular ultrafiltration, together with information concerning the sites and nature of certain of the tubular processes. The subsequent development by Smith [*3*] and his associates of suitable methods for estimating the volume of the glomerular filtrate by clearance technics has enabled the renal physiologist to attempt precise appraisals of the various tubular activities, both reabsorptive and secretory. The data collected over the past thirty years by micropuncture, clearance methods and certain recently developed technics, discussed elsewhere in this symposium [*4*], have provided a steadily growing body of knowledge concerning the quantitative aspects of renal function, the multiplicity of tubular transport processes, the sites within the nephron at which the various processes occur and, in some instances at least, an indication of the cellular activities which operate to accomplish tubular transport. Of particular importance to the present discussion is the observation that in many of the transfer processes solutes move across the tubular epithelium against a concentration gradient.

The present article will deal primarily with recent attempts to gain an understanding of the biochemical events which underlie the active transfer of certain compounds across the renal tubule. The problems posed are obviously not unique in the kidney. It has long been recognized that an indispensable property of every living cell is the capacity to establish and maintain concentration gradients across the cell membrane. The unequal distribution of sodium and potassium ions between the extracellular and intracellular compartments of skeletal muscle provides a classical example. Furthermore, it is now well established that such concentration gradients are not reflections of a selective permeability of the membrane, but are dependent upon the continuous metabolic activity of the cell.

DIFFUSION AND TRANSPORT

In dealing with the general problem of transport, a brief consideration of certain terms may be useful. A satisfactory definition of active transport has proved to be difficult, as has been pointed out by Rosenberg [*5*], Danielli [*6*] and others. It is generally accepted that in the absence of filtration, substances move across biological membranes by *simple diffusion*, *facilitated diffusion*, *active transport*, or by a combination of these processes. *Simple diffusion* signifies that the

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movement of molecules occurs along a concentration gradient by thermal agitation and that it conforms to Fick's laws of diffusion. Examples of simple diffusion have been described in biological systems, such as the penetration of ethanol into the human erythrocyte. However, when attention is directed to most of the compounds of biological interest, observable divergences from the laws of simple diffusion are common. This should not be surprising when it is appreciated that Fick's equations apply to ideal limiting conditions and that the conditions within the cell membrane are still largely unknown. The term *facilitated diffusion* has been used to describe certain of these exceptions. Again, movement occurs along a concentration gradient to reach the same equilibrium as in simple diffusion, but steric factors appear to be of importance; the unequal rates at which isomeric hexoses penetrate the erythrocyte is an example. In facilitated diffusion it is assumed that the membrane contains "active sites" at which the penetrating substance may combine with some cellular component to alter its chemical properties and therefore its rate of diffusion through the complex structure of the membrane. A specific molecular configuration probably underlies the capacity to combine, and accounts, as in the present example, for the facilitated diffusion of some hexoses and not of others. The role of diffusion in the movement of certain weak acids and bases across the renal tubule is considered in Dr. Milne's article in this symposium.

The term *active transport* is generally applied only to those systems in which a substance is transported across one or more biological membrane against a concentration gradient (uphill transport) at the expense of energy derived from the metabolism of the cell. Several limitations of this definition should be emphasized. First, active transport undoubtedly operates in some systems in which the establishment of a concentration gradient is not readily demonstrable. Since the bulk of glucose absorption from the intestine may occur at a time when the concentration of glucose in the intestinal lumen is higher than in the blood, diffusion may appear to make an important contribution. However, Auchinachie et al. [7] have shown that the viable intestinal mucosa actually imposes a considerable restraint on the passive diffusion of glucose. Moreover, the capability of the transport mechanism to move glucose in an uphill direction has been amply demonstrated both *in vivo* [8] and *in vitro* [9]. It appears probable that active transport participates in the movement of virtually all the glucose that crosses the intestinal mucosa and that diffusion processes are only of subsidiary importance. A second difficulty that arises in defining active transport relates to the dependence on metabolic activity. The demonstration that a transport mechanism can be inhibited by unfavorable metabolic conditions, such as anoxia or selective enzyme inhibition, provides only suggestive evidence of the active nature of the transport. It should be remembered that the maintenance of cellular structure is also dependent on continuous metabolic activity and that subtle changes in membrane structure, for example, could modify diffusion processes as well as active transport.

A fairly large number of transport processes fulfilling the requirements mentioned have now been studied, and certain additional features more or less common to all these systems can be described. First, active transport is *unidirectional*, i.e., each transport system appears always to move a given substance in a single direction. The demonstration that certain substances are both reabsorbed and secreted by the renal tubule, as with potassium ions, does not refute this. The available evidence indicates that potassium reabsorption occurs in the proximal tubule and potassium secretion at some more distal site in the nephron, that is, by different transport mechanisms. Second, many active transport systems exhibit a *non-linear relationship between concentration and the rate of transport*. Although the rate of transport can be shown to increase as the concentration of material present for transport is increased, eventually saturation of the mechanism occurs and a fixed maximal transfer rate is attained. Such a maximal rate of transport in the kidney is designated as Tm. The manner in which Tm is approached in the kidney varies from system to system. In glucose reabsorption [10] or p-aminohippurate secretion [11], the affinity of the system for the transported compound appears to be great and transport is essentially complete up to the point of saturation. In contrast, appreciable quantities of filtered glycine [12] escape reabsorption long before the level of saturation is reached, and Tm is attained only at load/Tm ratios of 1.5 or higher. Wilbrandt [13] has attempted to apply the kinetics of enzyme reactions to such renal transport systems and has found that the physio-

logical data conform quite well to a modification of the Michaelis-Menten equation, i.e., the equation commonly employed to describe the interaction of enzymes and substrates.

The attainment of a stable maximal rate of transport has been interpreted by Shannon [*14*] to indicate that active transport involves the reversible combination of the transported compound with a "cellular element" or "carrier" present in a constant, but limited amount. The demonstration of competitive phenomena, another common feature of active transport systems, is in keeping with the concept of a cellular carrier of limited capacity. Thus, when two or more compounds share the same transport mechanism, it may be expected that each will tend to displace the other as saturation of the mechanism is approached.

The exact meaning of the term "carrier" remains obscure, for neither its chemical nature, its site within the cell, nor its manner of combination with the transported compound can be described for any known transport system. In the discussion that follows, particular attention will be given to two representative renal transport systems: (1) glucose reabsorption, and (2) the tubular secretion of p-aminohippurate and related compounds. This arbitrary limitation to systems which transport organic compounds may seem unduly restrictive, especially when it is recognized that the major tasks of the kidney are concerned with the transport of the strong electrolytes. The latter, however, are dealt with in other contributions to this symposium. The biochemist's familiarity with the reactions of organic compounds in metabolic processes and with many of the pertinent enzymes accounts in part for the attention which the transport of organic compounds has received. It has seemed reasonable to expect that the relative abundance of information concerning glucose metabolism should be helpful in revealing the details of glucose transport. A different situation obtains in the case of tubular secretion, for in this instance we are dealing with a group of compounds which have no known fate other than excretion. Consequently, any observable metabolic reactions in which they participate within the cell may be suspected to be related to the transport process. It can be stated at the outset that the information acquired thus far is fragmentary and inadequate to describe either of these systems in precise biochemical terms. However, a sampling of the accumulated experience may be of interest if it serves to delineate more clearly the nature of the problem and to reveal some of the limitations of current approaches.

GLUCOSE REABSORPTION

Physiological Aspects. The reabsorption of glucose across the renal tubular epithelium appears to have all the qualities of an active transport process. Micropuncture studies in necturus, frog, guinea pig and rat [*2,15*] have shown the concentration of glucose in the glomerular filtrate to be the same as that in the plasma water. Samples of tubule fluid obtained at successively lower levels reveal a progressive decline in glucose concentration, so that glucose reabsorption is virtually complete at the midpoint of the proximal convolution. It is evident that glucose is reabsorbed in advance of water and electrolytes and that it is being returned to the peritubular fluid against an ever increasing concentration gradient. In the phlorizin-treated rat, the concentration of glucose in the tubular urine was increased and U/P ratios as high as 3 were observed at the mid-point of the proximal segment. In the phlorizinized guinea pig, it is noteworthy that creatinine, which is neither reabsorbed or secreted by the tubules, and glucose were concentrated to the same degree. This observation indicates that negligible quantities of glucose could diffuse across the tubule, even though a concentration gradient favoring diffusion into the peritubular fluid had been created by the reabsorption of water and electrolytes. Additional evidence of the relative impermeability of the tubule to the passive diffusion of glucose has been obtained by other technics in the frog by Höber [*16*] and in the dog by Chinard [*17*]

Glucose reabsorption in the intact kidney has been examined in a variety of experimental circumstances by clearance technics. Stable maximal rates of glucose transport (Tm_G) are readily demonstrable in the dog [*10*] and in man [*18*]. As already noted, an analysis of the data by Shannon [*14*] led him to propose that glucose transport involves a sequence of two reactions: (1) a reversible combination of glucose with the hypothetical cellular carrier, and (2) dissociation or decomposition of the carrier complex. In this sequence, the second reaction appeared to be first-order in type and rate-limiting for the over-all process.

The specificity of the glucose transporting

mechanism is not well established. Galactose [*19*], fructose [*20*] and xylose [*21*] are all partially reabsorbed from the glomerular filtrate in the species in which these sugars have been examined. In the dog, not more than 70 per cent of filtered fructose or 20 per cent of filtered xylose is reabsorbed even at low plasma levels. When the plasma levels of these three sugars are raised to high levels, the quantity reabsorbed increases, but limiting rates of transport (Tm) have never been observed. Attempts to demonstrate mutual interference for reabsorption between glucose, fructose and galactose have not been conclusive, but a sufficient elevation of the plasma glucose to produce glycosuria blocks the reabsorption of xylose almost completely. The reabsorption of all four sugars is inhibited by phlorizin.

Biochemical Aspects. Speculations concerning the nature of glucose transport have necessarily reflected basic developments in physiology and biochemistry. The early elucidation of the metabolic pathways in fermentation and anaerobic glycolysis revealed the important roles of the hexose phosphates and focused attention on these compounds as possible intermediates in sugar transport, both in the kidney and intestine. Lundsgaard [*22*] in 1933 first demonstrated that phlorizin interfered with the formation of hexose phosphates in muscle extracts, a finding later confirmed by Kalckar [*23*] in kidney minces. Subsequent studies by Shapiro [*24*], and Lotspeich and Keller [*25*] have shown that phlorizin inhibits the oxidative reactions of the citric acid cycle and the generation of adenosinetriphosphate (ATP) which normally accompanies these reactions. In addition, the ability of phlorizin to inhibit the phosphatases that hydrolyze hexose phosphates is well known. The demonstration that kidney and intestine are capable of successively phosphorylating and dephosphorylating glucose, and that phlorizin can inhibit either of these processes, has provided the basis for a widely accepted scheme to account for glucose transport.

According to the "phosphorylation-dephosphorylation" theory, glucose penetrates the luminal membrane of the tubule cell by diffusion and, in the adjacent cytoplasm, is converted to glucose-6-phosphate at the expense of ATP (hexokinase reaction). The removal of free glucose by phosphorylation is regarded as a device for establishing a concentration gradient across the luminal membrane favorable to the continued inward diffusion of glucose. The second reaction of transport, hydrolysis of the hexose phosphate (phosphatase reaction), is presumed to occur at a site within the cell further removed from the lumen than that of phosphorylation. It is essential that the second reaction yields free glucose in a concentration sufficiently higher than that of the interstitial fluid to permit outward diffusion through the peritubular membrane.

Evidence directly supporting the concept that phosphorylation and dephosphorylation participate in the transfer of glucose in the kidney or intestine is lacking. Although alkaline phosphatase has been shown by histochemical technics [*26*] to be abundantly present in the region of the brush border of the proximal tubule, the enzyme so located cannot function solely in glucose reabsorption, for its presence has been demonstrated in the "proximal" segments of aglomerular species (goosefish, toadfish, pipefish) as well as in glomerular species [*27*]. With regard to the intestinal absorption of sugars, several investigators [*28–30*] have reported that the ability of intestinal mucosa homogenates to phosphorylate various sugars roughly parallels the absorption rates of these sugars.

Various lines of evidence seem inconsistent with the phosphorylation theory. Frequently noted are the discrepancies between the doses of phlorizin required to produce maximal glycosuria and the concentrations needed *in vitro* to inhibit phosphorylation and dephosphorylation mechanisms. Such discrepancies obtain even when it is assumed that the administered phlorizin localizes exclusively, but is uniformly distributed, in the kidneys. Of course, an even more specific localization at its site of action cannot be excluded. Another disturbing observation comes from the studies of Dratz and Handler [*31*], who injected radioactive orthophosphate into cats and subsequently followed the rate of turnover of glucose-6-phosphate in the kidneys. The turnover rate appeared to be very slow and was not significantly altered either by glucose loading or by the administration of phlorizin. In addition, Cori [*32*] has commented on the absence of glycosuria in von Gierke's disease, a condition in which histochemical technics reveal a striking deficiency of the specific glucose-6-phosphatase in the proximal tubule. Perhaps the most serious objection to the phosphorylation-dephosphorylation theory arises from the cytoplasmic nature of the

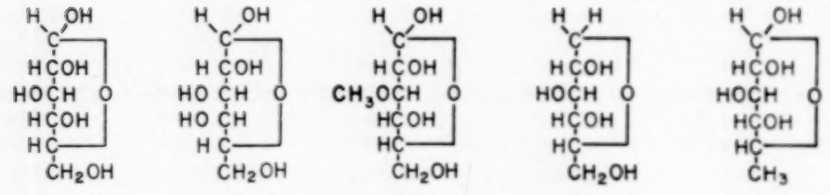


FIG. 1. Representative sugars actively transported by sacs of everted small intestine of the golden hamster. From: WILSON, T. H. and CRANE, R. K. The specificity of sugar transport by hamster intestine. *Biochem. et Biophys. Acta* [38].

proposed carrier. In the cytoplasm-carrier type of mechanism the cell membranes are presumed to be permeable to the transported compound, but relatively impermeable to the transport intermediate. Evidence that glucose does not readily penetrate the tubular epithelium by passive diffusion has already been cited. An additional apparent defect of the cytoplasm-carrier mechanism emphasized by Wilbrandt [13] is that it provides no barrier to the back diffusion of free glucose from reaction 2 to the site of reaction 1, so that appreciable quantities of glucose might be expected to recycle continuously through the two reactions at a considerable cost in efficiency.

Probably the most significant contributions to an understanding of glucose transport have come from recent studies on the intestinal absorption of sugars. Although it is not certain that similar mechanisms operate in the kidney and intestine, the same theory has been proposed to account for sugar transport in both organs. Two natural sugars, glucose and galactose, and a synthetic derivative, 3-methylglucose, are absorbed from the small intestine of the rat and other animals at rates considerably greater than are observed with any of the other sugars tested [33,34]. Sols [35], who has recently reappraised the substrate specificity of the hexokinase present in the intestinal mucosa of the rat, has found that neither galactose or 3-methylglucose is phosphorylated to an appreciable degree. Inadequate methods for the assay of hexokinase activity probably account for the earlier contrary reports. That all three of these sugars are actively transported by the intestine *in vitro* has been conclusively demonstrated by Wilson and Wiseman [9], and Wilson and Vincent [36]. Sacs of everted small intestine of the hamster or rat accumulate glucose, galactose and 3-methylglucose against significant concentration gradients. The accumulation process is dependent on aerobiasis and is inhibited by low concentrations of phlorizin or dinitrophenol. Crane and Krane [37], and Wilson and Crane [38] have examined a variety of sugars by this technic in an attempt to learn whether or not any specific molecular configuration is required for active transport. *D*-Glucose, *D*-galactose and 3-methyl-*D*-glucose are accumulated at relatively high rates; 6-deoxy-*D*-glucose and 7-deoxy-*D*-glucoheptose at intermediate rates; and 1-deoxy-*D*-glucose, 6-deoxy-*D*-galactose, *D*-allose and *D*-glucohepulose at somewhat lower rates. No active transport could be demonstrated with *D*-fructose, *D*-mannose, 2-deoxy-*D*-glucose, *L*-galactose, *D*-glucosamine, *D*-xylose or a number of other 5-, 6- and 7-carbon sugars. Some of the representative hexoses actively transported by the small intestine in the hamster are shown in Figure 1. From these studies, the authors have concluded that a generalization can be made regarding the specificity of the transport mechanism. All the sugars actively transported have in common a *D*-pyranose structure (1,5-oxygen bridge), a methyl or substituted methyl group attached to carbon-5, and a hydroxyl group in the glucose configuration at carbon-2. It is of particular interest that 6-deoxy and 1-deoxyglucose are transported, for these compounds exclude the possibility of phosphorylation at the sites previously considered to be of major importance in speculations on glucose transport.

Krane and Crane [40] have recently demonstrated that *D*-galactose, a sugar reabsorbed by the renal tubule and metabolized by kidney tissue at a low rate, is actively accumulated *in vitro* by slices of renal cortex from the rabbit. The accumulation occurs against a concentration gradient, is dependent on aerobic metabolism, and is inhibited by phlorizin, dinitro-o-

cresol or *D*-glucose, but not by *D*-xylose. It appears probable that the accumulation of galactose by kidney slices *in vitro* reflects the capacity of the renal tubule to reabsorb this sugar *in vivo*. Studies with phlorizin in this system appear to throw new light on the mode of action of this agent. When the active accumulation of galactose is blocked by dinitro-o-cresol, the concentration of galactose in the slices reaches an equilibrium value approximating 90 per cent of that in the surrounding medium, presumably by diffusion. The addition of phlorizin to the inhibited system slows the rate at which the equilibrium is attained, a finding which suggests that phlorizin does not act primarily on the active transport system, but that it may act at the cell membrane to limit the penetration of sugars by diffusion.

Attention may now be directed to other metabolic processes which have been considered in relation to glucose transport. The possibility that transport might involve the cleavage of glucose into 3-carbon units with subsequent recombination, or that it might occur via the reactions of the pentose-phosphate pathway, appears to have been excluded by the studies of Chinard et al. [*17*]. Glucose-1-C^{14} injected into the renal artery of the dog and recovered from the renal venous blood showed no redistribution of the isotopic labeling. It must be concluded that glucose is transported by the renal tubule as an intact 6-carbon unit.

The most novel mechanism yet proposed for glucose transport is that of Kesten [*41*], who has found that the kidney contains mutarotase, an enzyme which catalyzes the interconversion of the α- and β-forms of *D*-glucose, *D*-galactose, *D*-xylose and *L*-arabinose. Mutarotase is another in the growing list of enzymes that can be inhibited by phlorizin. According to Kesten's hypothesis, glucose in the glomerular filtrate is predominantly of the α-configuration. As the filtrate enters the tubule, the action of mutarotase is believed to yield some form, intermediate between the α- and β-configurations, which diffuses passively into the peritubular fluid. It is assumed that the abundance of this form is kept low in the arterial blood, and hence in the glomerular filtrate and peritubular fluid, by its prompt removal and utilization by tissues other than the kidney. The postulated mechanism would require no expenditure of energy by the kidney in glucose reabsorption, for mutarotation is a reaction approaching equilibrium and therefore is energy-yielding. It is difficult to conceive that such a mechanism could accomplish the complete reabsorption of glucose. Some of the findings already mentioned appear to be inconsistent with this theory. If galactose accumulation by kidney slices is related to the tubular reabsorption of this sugar, a passive process can be excluded by the fact that the accumulation occurs against a concentration gradient. Mutarotation obviously cannot account for the uphill transport of sugars observed *in vitro* with the intestinal mucosa.

TUBULAR SECRETORY MECHANISMS

Physiological Aspects. Many substances are added to the urine by tubular secretion, that is, by transport from the peritubular fluid into the tubular lumen. Clearance studies in various species have served to identify several independent transport mechanisms, one being responsible for the secretion of a variety of weak organic acids, another for several organic bases and tetraethylammonium ion, and still others for creatinine, uric acid (birds and reptiles) and urea (frog) [*42*].

The mechanism studied most extensively is that which secretes the various weak organic acids, such as the phenolsulfonphthalein dyes, p-aminohippurate, diodrast®, penicillin, and certain glucuronides and ethereal sulfates. Studies in several species indicate that the transport process is limited to the proximal convolution. Maximal rates of transport (Tm) have been demonstrated with phenol red [*43*], diodrast [*44*] and p-aminohippurate [*45*], and the ability of the various compounds to compete with one another for transport provides convincing evidence that they share a common secretory mechanism. The physiological significance of this transport mechanism remains obscure, for most of the compounds handled by it are foreign to the body. Sperber [*46*] and others have emphasized its possible importance in the removal of "detoxication products," and evidence is accumulating that it may constitute an important route for the excretion of certain biological conjugates, such as the glucuronides of adrenal steroid metabolites [*47*].

Biochemical Aspects. The advantages of this system for biochemical studies on transport are several-fold. The transport system appears to be present in all the vertebrates, thus permitting valuable comparative biochemical correlations. Most of the compounds transported are des-

tined only for excretion and, therefore, presumably do not enter into metabolic reactions unrelated to transport. Finally, the secretory mechanism can be studied quantitatively both in the intact kidney and in *in vitro* preparations.

The *in vitro* preparations which have proved to be of value in these studies include tissue cultured cystic explants of the chicken mesonephros [*48*], the isolated renal tubules of various fishes [*49,50*] and slices of mammalian kidney cortex [*51,52*]. In each of these preparations the accumulation of the transported compound against a concentration gradient is readily demonstrated. In isolated tubules of the flounder, phenol red accumulates within the tubular lumen in concentrations which may reach values several hundredfold that in the surrounding medium. No staining of the tubular epithelium is apparent and it seems unlikely that storage within the cell is of quantitative importance. Slices of mammalian kidney cortex differ in that the accumulation of the dye appears to be predominantly intracellular, but is limited to the cells of the proximal tubular segment [*53*]. This apparent defect of *in vitro* mammalian preparations cannot be explained, but its occurrence suggests that the secretory mechanism involves at least two distinct and separable processes, (1) the movement of dye from peritubular fluid into the cell, and (2) movement from the cell into the tubular lumen. There is no reason for suspecting that the intracellular accumulation is an artefact unrelated to transport, for most of the phenomena which have been demonstrated by this technic have proved to be applicable to function in the intact kidney as well.

The tubular secretory mechanism is completely dependent upon aerobic metabolism, being blocked by anaerobiasis, by inhibitors of the cytochrome-cytochrome oxidase system of electron transfer, or by inhibitors of the oxidative reactions of the tricarboxylic acid cycle [*51,54*]. This dependence on aerobic metabolism appears to be related to the requirement of the transport system for energy-rich phosphates, such as ATP, which are generated by these oxidative reactions. Dinitrophenol and other agents which "uncouple" aerobic phosphorylation, i.e., block the generation of ATP without depressing respiration, have been shown to inhibit the secretory mechanism both *in vitro* [*50,55*] and *in vivo* [*56*]. The manner in which phosphate bond energy is utilized in transport remains obscure, but several of the more obvious possibilities merit comment. First, there is no evidence to suggest that any of the transported compounds undergoes reversible phosphorylation. It would appear much more likely that the hypothetical cellular carrier is the element of the transport system which must be raised to some more reactive state at the expense of phosphate bond energy. Finally, as mentioned earlier, interference with energy transfers within the cell may produce structural alteration which could account in part, but not entirely, for the observed effects.

The search for an appropriate cellular carrier in this system can be dealt with most conveniently in a chronological account. Early experiments [*51*] revealed that certain normal metabolic intermediates can exert profound effects on the transport of p-aminohippurate. The most striking was the finding that low concentrations of acetate are capable of doubling the rate of PAH transport in kidney slices from the rabbit. This effect was confirmed by clearance technics in the dog with the demonstration that the Tm of PAH could be raised abruptly by intravenous infusions of acetate [*57*]. By contrast, PAH transport is markedly inhibited by low concentrations of the dicarboxylic acids of the tricarboxylic acid cycle, by certain amino acids (L-glutamate and L-alanine) and by the fatty acids of intermediate carbon chain length (C_5-C_{10}). Neither the augmentation nor depression of transport by the various substrates can be related to effects on cellular respiration or changes in acid-base balance.

It appeared reasonable that a clarification of these substrate effects would provide information concerning the nature of the transport mechanism. The apparently unique stimulatory effect of acetate suggested the possible participation of acetyl-coenzyme A as an important rate-limiting cellular component of the system. Largely on this basis, it was proposed [*58*] that acetyl-CoA might represent the activated carrier and that PAH transport might involve an acyl-exchange reaction to yield p-aminohippuryl-CoA as the transport intermediate. Subsequent studies of coenzyme A-dependent systems in the kidney, however, provided an alternative explanation for the acetate effect [*59*]. The kidney was found to contain enzymes capable of synthesizing a variety of acyl-glycine conjugates. Acetate and the fatty acids are converted in the presence of ATP, CoA and the appropriate

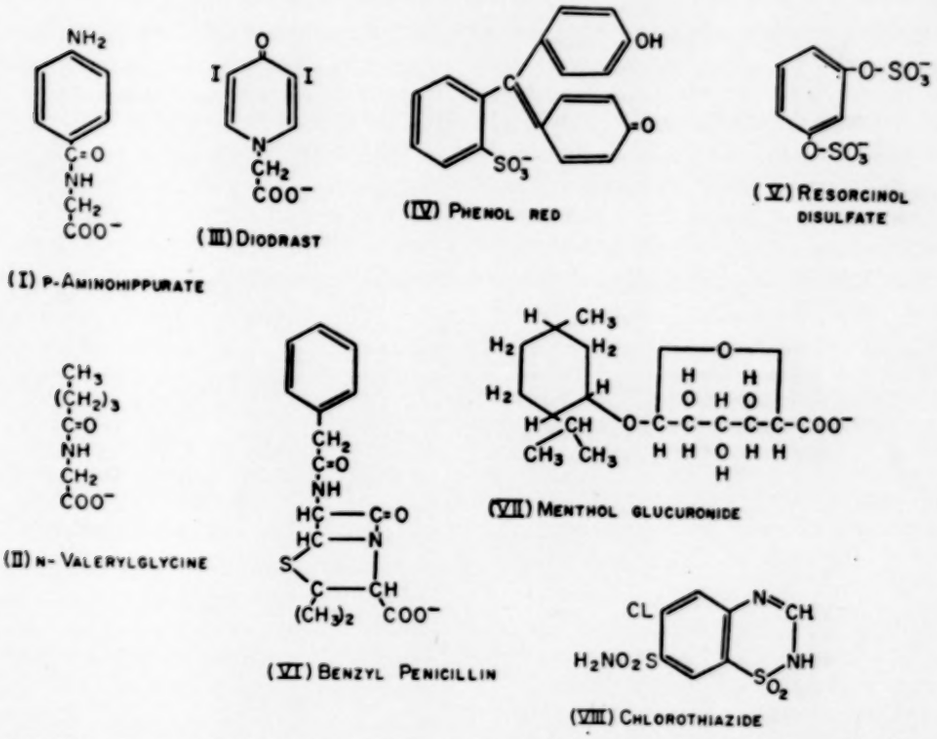


FIG. 2. Some representative compounds actively secreted by the renal tubule by a common excretory mechanism.

activating enzymes into the corresponding aliphatic acyl derivatives of CoA. These in turn react with glycine, under the influence of the enzyme glycine-N-acylase, to yield the corresponding aliphatic acylglycines. The structural similarity of these compounds to p-aminohippurate, an aromatic acylglycine, prompted a study of their accumulation in kidney slices and an appraisal of their ability to compete with PAH for transport. It was found that acetylglycine, owing to its instability in the kidney, neither accumulates nor competes. Propionylglycine and butyrylglycine are weak competitors, but when the acyl group is lengthened to 5 or more carbons the aliphatic acylglycines are actively accumulated and become potent competitors with PAH for transport. These observations can be interpreted to account for both the stimulatory effect of acetate and the inhibitory effects of the fatty acids of intermediate chain length. The latter simply give rise to competitive acylglycines. It is probable that PAH transport, at least as observed in kidney slices or in the intact animal at Tm levels, is always partially inhibited by certain aliphatic acylglycines arising from the acyl-CoA's present in the main channel of fatty acid oxidation and synthesis. The addition of acetate to the system should yield acetyl-CoA in relative abundance and should promote the synthesis of acetylglycine at the expense of the longer chained inhibitory acylglycines. Since acetylglycine is not effective as a competitor, the net effect of adding acetate is to remove an endogenous inhibition of transport. This hypothesis is supported by the finding that acetate stimulates PAH transport only in those species which possess the glycine conjugation system; acetate has no effect in the species lacking this synthetic capacity. Although the studies relating to coenzyme A have provided plausible explanations for some of the substrate effects, they shed little light on the nature of the transport mechanism. They emphasize, however, the manner in which unrelated metabolic activities of the cell may influence the transport process.

Figure 2 presents the structures of representative compounds which share the common secretory pathway. Compounds I to VII can be classed in two groups: the carboxylic acids (p-aminohippurate, n-valeryl-glycine, diodrast, penicillin and menthol glucuronide) and the sulfonic acids (phenolsulfonphthalein and resorcinal disulfate). It has been assumed that the anionic group

(—COO^- or —SO_3^-) is of primary importance in whatever interaction occurs between the transported compound and the cellular carrier. Chemical substitution of the carboxyl group in PAH, as in p-aminohippurhydroxamate, has been shown to abolish transport, whereas substitutions on other potentially reactive groups have negligible effects [*60*]. In an attempt to determine the nature of the chemical bonding involved, PAH labeled in the carboxyl group with O^{18} has been utilized [*60*]. The labeled material was infused into the renal artery of a dog and recovered from the urine. Transport of the PAH across the tubular epithelium resulted in no loss of O^{18} from the carboxyl group. This finding excludes several important types of covalent linkage between the carboxyl group and carrier, for either their formation (amide, thiol ester) or subsequent hydrolysis (ester) would lead to a loss of O^{18} from the carboxyl group. A mixed carboxylic-phosphoric anhydride (—CO—O—P—), such as occurs in the acyladenylates, remains a possibility and Kellerman [*61*] has recently shown that benzoyl- and hippuryladenylates can be prepared synthetically, and subsequently hydrolyzed by a renal enzyme in such a manner that the original carboxyl oxygens would be retained. However, no evidence has been obtained to indicate that the kidney is capable of forming acyladenylates of any of the transported compounds.

Beyer's recent observation [*62*] that chlorothiazide (VIII, Fig. 2) is secreted by the renal tubule is of particular interest, for this compound contains neither a free carboxylic nor sulfonic acid group. Studies of the accumulation of chlorothiazide by kidney slices in Beyer's laboratory and in our own provide quite convincing evidence that chlorothiazide is transported by the same secretory mechanism. If this conclusion is correct, one can apparently dismiss from future consideration any mechanism which entails a covalent bonding between the cellular carrier and a carboxylic or sulfonic acid group.

Höber [*63*], in reviewing his experiences with a series of sulfonate dyes, noted some years ago that the transported dyes have a bipolar structure, one end of the molecule being predominantly hydrophilic and the other organophilic. It is worth noting that the structures presented in Figure 2 still appear to share this feature with Höber's original dyestuffs. This is particularly true of the series of aliphatic acylglycines (*cf.* II, Fig. 2) in which transport does not become evident until the aliphatic group is sufficiently long to acquire an organophilic-hydrophobic character. The physiological significance of this bipolar configuration is uncertain, but Höber has suggested that it may be of importance in orienting the molecule at the cell surface prior to its entry into the active transport system.

SUMMARY

It is evident from the present review that we are still far removed from a real understanding of the nature of active transport processes. The manner in which metabolic energy can direct the orderly movement of molecules across biological membranes against an electrochemical potential remains obscure. It is probably significant that the experiences with two representative transport systems have led to similar conclusions. In these instances, at least, the application of current biochemical concepts and technics has served to eliminate many of the mechanisms proposed in the past on rather superficial grounds. The rapidly growing body of information in this field should aid in the formulation of future research efforts which may be expected to shed new light on the basic mechanisms involved.

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Clinical Patterns of Tubular Dysfunction*

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SINCE the time of Richard Bright's paper in 1827 it has been customary to associate the term "chronic renal insufficiency" with the clinicopathological findings described by him. Nitrogen retention, albuminuria, metabolic acidosis and anemia are commonly observed. Even though the pathogenesis of each of these manifestations has not been completely elucidated, there is general agreement that they are associated with failure of total renal function, i.e., a decrease in the number of normally functioning nephrons with a corresponding fall in filtration rate and depression of the various tubular functions. Chronic glomerulonephritis, arteriolar nephrosclerosis and pyelonephritis are the most common causes, but many other diseases lead to essentially the same pattern of uremic renal insufficiency.

Within the past several decades increasing attention has been focused upon a variety of syndromes which contrast with the condition described by Bright. Some of these are quite rare but all appear to have in common a type of chronic renal insufficiency that is characterized primarily by defects in one or more tubular functions. Although albuminuria and nitrogen retention are usually absent, variable degrees of glomerular insufficiency may eventually develop, apparently as a consequence of general metabolic disturbances secondary to the tubular defect. However, even in these instances glomerular insufficiency is not necessarily marked and the disease picture is dominated by the tubular abnormality.

Current interest in these disorders stems largely from three considerations. First, many tubular defects fall under the category of "reversible renal insufficiency." This is not to imply that the primary defect necessarily can be corrected, but rather to emphasize that the results of proper replacement therapy are often dramatic. The management of chronic uremia has always represented a rather dismal area in therapeutics. In contrast, even though tubular defects may lead to rather striking physiological derangements, appropriate therapy may often be undertaken with optimism since the specificity of the defect implies a normal reserve for other renal functions. Second, these syndromes present a unique opportunity for the study of renal mechanisms. While the experimental physiologist has been able to describe many aspects of tubular function, it is not unreasonable to believe that future studies of spontaneous disease may be more productive than controlled laboratory experiments. And third, this group of disorders has become an area of major interest to medical genetics. For case finding studies, an abnormality in renal function may provide a readily identifiable "marker" which can be detected by rather simple chemical examination of either the blood or urine. Many studies of inborn errors of metabolism have emphasized the abnormal events in a sequence of intracellular metabolic reactions; these errors may also be reflected as abnormalities of active transport. The renal tubule constitutes one of the few organs in which transport across an epithelial membrane can be studied in the intact subject by accurate and quantitative technics.

It is of some historical interest that one of the first abnormalities to be described was that of uric acid excretion in the Dalmatian coach hound. The student of medicine has long been familiar with the fact that an abnormality in urate metabolism occurs in this breed [*1*] but it was not until the study of Friedman and Byers in 1948 [*2*] that the nature of the metabolic defect was clarified. It had previously been held that increased urate excretion was attributable to its failure to be converted to allantoin. Studies of renal excretion showed that non-Dalmatian dogs excreted about one-fourth of the filtered urate, while the Dalmatian excreted all of it. When urinary excretion was prevented by ureteral ligation, the subsequent changes in

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plasma levels indicated that the Dalmatian was able to convert approximately 75 per cent of the retained urate to allantoin. Thus the primary metabolic abnormality is a tubular defect in urate reabsorption. Subsequent studies have indicated that this is transmitted by a single recessive gene [*3*]. Our concepts of essential cystinuria in man have followed a similar course; a metabolic derangement of cystine metabolism was formerly considered to be the underlying mechanism until the studies of Dent [*4*] demonstrated a renal tubular defect.

Parenthetically, the frequency of the various tubular disorders in man appears relatively high compared to their known incidence in laboratory animals, but this is probably simply a reflection of the number of studies reported. Cystinuria in canines has been described as a genetically determined variant, but quantitative studies on renal mechanisms are not available [*5*]. Some strains of mice excrete exceptionally large amounts of taurine at normal plasma levels, suggesting an inheritable variant of renal function [*6*]. Further studies in suitable laboratory animals might clarify many problems by the use of direct technics that cannot be applied to man.

In classifying defects of tubular function a reasonable point of departure might be to consider the known functions of the tubule and then to list corresponding instances of dysfunction. However, this would not necessarily be a realistic approach because of the tremendous overlap in the observed clinical syndromes. For example, Dent [*7*] has reported two siblings with renal hypophosphatemia. While various genetic considerations would indicate that both were suffering from the same disease, on further examination it was found that one patient had renal glycosuria but the other did not. Were these varying manifestations of a single underlying disorder or were they two separate and distinct renal abnormalities? Obviously, rigid classifications are in themselves unproductive. Nevertheless, it may serve some purpose to emphasize both the number of observed syndromes as well as the apparent defects of individual functions.

A variety of different mechanisms may be envisaged by which a genetic variant becomes manifest as an abnormal pattern of urinary excretion. (1) If the trait expressed itself as a block in a sequence of extra-renal metabolic reactions, this would result in an increase in the concentration of metabolic intermediates in body fluids, which might consequently be excreted in increased amounts by the kidney. As an example, the defect in phenylketonuria is characterized by a marked elevation in the plasma concentration of phenylalanine with an approximately proportional increase in its rate of excretion in the urine [*77*]. This is an example of an "overflow" type of phenomenon, giving rise to an increase in total excretion and representing a normal renal response to an increased load. (2) The variant might express itself as an abnormality of tubular transport. In the case of impaired reabsorption, this would result in excessively high clearance of the substance under consideration (see for example, essential cystinuria). (3) The genetic defect, while having a direct primary action on a single renal transport system, might so alter normal homeostatic mechanisms that other tubular functions would eventually be jeopardized. Although not established unequivocally, interactions of this type may be involved when multiple transport systems appear to be affected by a single genetic variant. (4) The trait might involve an extra-renal metabolic reaction and give rise to an increase in plasma concentration and the rate of excretion of an abnormal metabolite, and this, in turn, might impair the tubular transport of an apparently unrelated substance. The renal aminoaciduria of galactosemia has been attributed to this sequence of reactions. And, (5) Harris [*9*] has suggested the possibility that certain diffuse disorders may be attributed to a defect in the active transport systems of more than one organ. Thus, if a single cellular component were required for transport across the renal tubule as well as other cellular membranes (blood-brain barrier, for example), the defect might be most obvious in the pattern of urinary excretion because of the ease with which renal function may be evaluated, but similar events might be occurring in less accessible tissues. While there is no unequivocal example of this, Harris suggests the complex manifestations of Hartnup's disease as a possibility.

The technics of genetics may be productive in describing not only the patterns of inheritance but also the functional attributes of certain processes. Studies of essential cystinuria have suggested a common reabsorptive mechanism for lysine, cystine, ornithine and arginine [*10*]. In addition, on the basis of the behavior of presumed heterozygotes, it has been postulated that the transport mechanism has a greater affinity for arginine and ornithine than for

cystine and lysine. However, caution is advised against too rigid an application of genetic principles. It is often impossible to distinguish between primary phenotypic manifestations and secondary metabolic derangements, and it should not be presumed that all tubular syndromes are inborn errors. Heavy metal poisoning, in particular, may mimic a variety of congenital defects. Infections are common sequelae, especially when the primary disorder leads to nephrolithiasis or nephrocalcinosis. Although there is no known specific tubular defect which has been proved to be the result of a primary infection, there is no *a priori* reason for excluding this as a possibility.

At the present time the major difficulty in the evaluation of an abnormal pattern of urinary excretion is to determine whether it is of the "overflow type" or primarily renal in origin. The following possibilities arise in consideration of quantitative measurements: (1) *The overflow type of increased excretion.* This is not a primary tubular defect and is associated with an extrarenal disturbance giving rise to abnormally high plasma levels. Although total excretion may be increased, renal function may be considered unimpaired since the renal clearance of the metabolite is normal in relation to the increased load. (2) *Increase in renal clearance with increase in total excretion.* This is the most common relationship encountered in the tubular disorders in man. An increase in renal clearance (UV/P) might result from an increase in the amount excreted (UV), or from a decrease in the plasma concentration (P), or from a combination of both. Often, the most difficult analytical problem is the accurate measurement of plasma concentration, and it is this value which may be most critical in establishing the nature of the disorder. (3) *Increase in renal clearance with normal rate of total excretion.* This is the simple consequence of having UV remain constant in the presence of decreased values for P. If syndromes of this type were considered only in terms of total excretion, there would be no apparent renal abnormality. However, renal function is definitely impaired since the normal tubule responds to a decrease in the plasma concentration of most substances by a reduction in the amount excreted. This normal response may lead to eventual restoration of more normal plasma values, and thus the kidney serves a useful role in homeostasis. The primary consequence of this type of tubular defect is related to the low plasma concentration itself. In most instances an abnormally high clearance is associated with a substance that is excreted by filtration and selective reabsorption. Supranormal filtration rates or a significant decrease in plasma binding have not been reported, and therefore an increased clearance can usually be attributed to a decreased rate of reabsorption.* With other substances which are excreted by tubular secretion, an increase in clearance would denote an increase, rather than a decrease, in the rate of tubular transport. The various forms of excessive potassium excretion may fall in this category.

The application of modern technics of renal physiology has focused attention on two possible interpretations of the general nature of tubular defects. Renal glycosuria has probably been investigated more thoroughly than any other condition and may therefore be used for illustration. However, the same concepts are probably applicable to other syndromes and also to both reabsorptive and secretory transport. Renal glycosuria is characterized by (1) the appearance of glucose in the urine at either normal or even hypoglycemic levels of plasma glucose and (2) the ability of the kidney, as a whole, to increase glucose reabsorption when the filtered load is elevated. The question may therefore be asked—Why does glycosuria appear at normal blood levels (i.e., decreased threshold) when the maximum capacity to reabsorb glucose (i.e., Tm) is only achieved during hyperglycemia? The two concepts that have been mostly considered may be identified as involving either the "nephron population" or the "kinetic aspects of the transport reactions."

The curves of Figure 1 summarize certain quantitative relationships between load and transport. These are measurements of the total function of both kidneys. The line labelled as "theoretical" would be obtained if two conditions determined the maximal rate of transport. First, it is required that the nephron population would be homogeneous to the extent that below maximal capacity transport would be propor-

* Theoretically, a tubular "defect" might consist of an abnormally *high* rate of tubular reabsorption which would eventually be characterized by an elevation in the plasma level. Clear-cut examples of primary renal defects of this type have not been definitely established. The findings in pseudohypoparathyroidism with elevation of plasma phosphate and the absence of the phosphaturic response to parathyroid hormone suggest an abnormally high phosphate reabsorptive capacity [*34*]. Detailed studies on renal mechanisms are not available.

tional to load in any single nephron. In reabsorptive transport, this would mean that glomeruli with high filtration rates would be attached to tubules with a large reabsorptive capacity, and similarly, small glomeruli would be associated with small tubules.* The events occurring within individual nephrons would also be described by the theoretical curve. As the amount available for reabsorption was increased, there would be a proportional rise in the amount reabsorbed until that point was reached at which the maximum transport capacity (Tm) of the nephron was saturated; beyond this point none of the further increase in load would be reabsorbed. If the balance between glomerular and tubular activity were the same in all nephrons, i.e., a homogeneous population, then the reabsorptive capacity of each nephron would be achieved at the same blood level, and the "titration curves" for the entire kidney would be of the same shape as the curve for the single nephron. The theoretical curve would therefore describe a population of nephrons that are homogeneous in respect to glomerulotubular balance. If, however, the reabsorptive capacity of some tubules was saturated at a relatively low blood level, glucose would be excreted in the urine derived from the tubules with the low "threshold." The titration curve for the entire kidney would deviate from the theoretical and the degree of deviation, or splay, would be proportional to the degree of heterogeneity.

In this consideration, it is assumed that no glucose is excreted from an individual nephron until the maximum rate of reabsorption for that nephron has been saturated. This involves the second condition that determines the theoretical curve. By application of simple concepts of kinetics, it is apparent that the maximal rate of transport across an individual cell might be achieved only when the substrate concentration (or load) was considerably greater than the Tm. Under these conditions, glucose would be excreted by the tubule at loads less than those required for complete saturation of the cellular mechanism. This would result in a splay in the titration curves, both for individual nephrons and for the kidney as a whole. In the normal kidney variations in the kinetic component depend primarily on the transport system under consideration. In the dog, there is only an insignificant degree of splay for p-aminohippurate (PAH), phosphate and sulfate [*12–14*]. For glycine, however, maximal transport capacity is only gradually attained, with considerable splay in the curve [*17*]. For other substances, notably sodium and chloride, the results with the loading type of experiment fail to disclose a maximal transport rate. A detailed analysis of the kinetics of tubular transport has been reported by Wildbrandt [*19*].

These findings in the normal animal suggest that the kinetic component of individual tubular mechanisms may be overwhelmingly more important than the possible heterogeneity of the population. An alternative explanation would be to attribute all these phenomena to variable degrees of glomerulotubular imbalance and virtually to ignore the kinetic component entirely. Thus, in the normal subject the nephrons might be relatively homogeneous with respect to glucose reabsorption, much less so with respect to glycine, and very heterogeneous with respect to sodium and chloride. However, there is good experimental evidence that documents the importance of the kinetic factor. In the dog, dinitrophenol inhibits the transport of PAH but has no effect on the reabsorption of glucose [*12*]. Titration curves for glucose and PAH in the normal dog are not significantly different from the theoretical curve in Figure 1. With dinitrophenol, the glucose curve remains unchanged, but the splay in the PAH curve increases enormously. This combination of results strongly indicates that the configuration of the titration curve primarily depends on the kinetics of tubular transfer reactions rather than upon variations in the function of individual nephrons.

These same considerations apply in the evaluation of spontaneous tubular defects. In renal glycosuria, the appearance of glucose in the urine at plasma levels far below those required to saturate the total reabsorptive capacity could be explained on the basis of a population of heterogeneous nephrons. Tubules with a diminished reabsorptive capacity would excrete glucose at a low plasma level which was insufficient to saturate the more normal nephrons. However, the same phenomena may be interpreted with equal validity as a diffuse defect involving all tubules; in renal glycosuria the reabsorptive mechanism may respond to an

* For reabsorptive mechanisms, loads are calculated as the product of plasma concentration and filtration rate; for secretory processes, as the amount delivered by the renal blood flow to the tubules. The same general considerations apply to each type of load.

increase in load in a manner similar to the PAH system when inhibited by dinitrophenol. It is also possible that both types of defects may exist in varying combinations.

Renal Glycosuria. Many features of this syndrome have already been discussed. By definition, it represents the occurrence of glycosuria at normal blood glucose levels. The condition is essentially benign and may be inheritable. Although in a few patients diabetes mellitus is said to have developed, the factors of coincidence cannot be rigidly excluded. Normal filtration rates are reported [*18,20,21*] and there is no evidence of progressive renal failure, such as may occur in other tubular syndromes. The defect is limited to the transport of glucose. The reabsorption of phosphate [*22*] and amino acids [*23*], and the secretion of PAH [*18*] are all normal. These several functions are attributed to the proximal tubule. As has also become evident for other tubular syndromes, a deficiency of one reabsorptive mechanism may occur in the same cell or segment in which other transport reactions are completely normal. In the studies of Reubi [*18*] and of Bradley [*21*] it was originally proposed that there were two types of tubular defects, both characterized by a low threshold, but one with a relatively normal glucose Tm, the other with a markedly decreased value. Re-evaluation of seventeen reported cases showed a spread of values which did not clearly fall into two separate categories [*8*]. Glucose titration curves have shown a pattern varying from a normal degree of splay to the marked abnormality depicted in Figure 1. The nature of the fundamental disturbance remains obscure.

Nephrogenic Diabetes Insipidus. This is a well defined disorder characterized by the failure of the tubule to respond to antidiuretic hormone (ADH), of either endogenous or exogenous origin. It occurs in most pronounced form in young infants who may pass excessively large volumes of urine and who are consequently subject to severe dehydration. In some patients high fever, without apparent infection, results from dehydration itself [*24,25*]. If water intake is adequate, the subjects may thrive in a normal fashion. However, frequent instances of stunted growth have been reported and are presumed to result either from decreased caloric and protein intake or as a direct consequence of chronic hyperelectrolytemia. Mental retardation has also been attributed to hyperosmolality. It has been proposed that rational management includes not only a plentiful intake of water, but also a restriction of solute intake, particularly by limitation of sodium chloride [*26*].

The nature of the renal abnormality is indicated by a variety of observations, all of which are consistent with failure of the kidney to

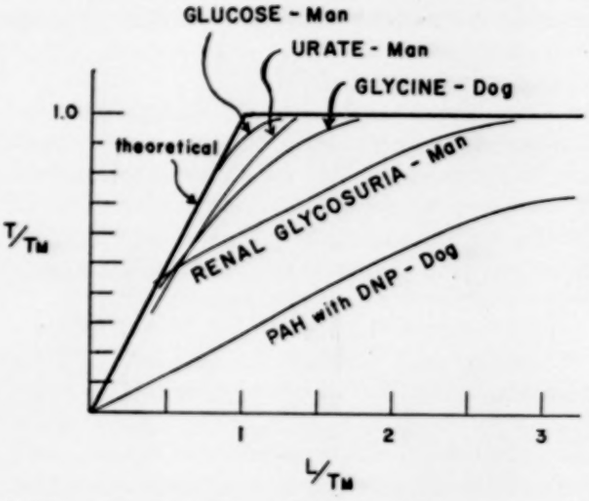


FIG. 1. Schematic representation of different types of "titration curves" for substances having a maximal rate of tubular transport (Tm). For discussion see text. The "theoretical curve" is indicated by the heavy line, and also approximately describes the available data for glucose [*11*], *p*-aminohippurate (PAH) [*12*], phosphate [*13*], and sulfate [*14*] in the dog. References for other curves are: glucose in man [*15*], urate in man [*16*], glycine in the dog [*17*], renal glycosuria in man [*18*], and PAH under the influence of dinitrophenol (DNP) in the dog [*12*].

respond to ADH in a normal manner. Glomerular filtration rate and renal blood flow are usually normal. The Tm's of glucose [*27*] and PAH [*24*] are not remarkable, although one adult had a diminished capacity to transport diodrast [*27*]. Glycosuria, albuminuria and aminoaciduria are absent; the renal response to an acid load is normal, as is the mechanism of sodium conservation [*28*].

The release of ADH by the posterior pituitary is believed to be unimpaired. There is no clinical evidence of lesions in the hypothalamic area. Rat bioassay of the urine reveals normal excretion of antidiuretic substances [*24*] but it would be desirable to re-evaluate this point with the more reliable assay in the dog with surgical diabetes insipidus [*29a*]. Williams and Henry [*27*] studied the plasma of their patient in an effort to detect an abnormally high rate of ADH inactivation; the results were negative.

The inability to concentrate the urine in

response to active preparations of parenterally administered vasopressin is the single feature which most decisively distinguishes this syndrome from diabetes insipidus of pituitary origin. However, failure to respond to vasopressin obviously cannot be accepted as pathognomonic, since a similar response may be obtained in other renal diseases, particularly when the relatively insensitive and inaccurate clinical determination of specific gravity is used as the sole criterion. The polyuria of many other tubular syndromes has been attributed to refractoriness to ADH but definite conclusions cannot be drawn until studies have been made with more refined technics including the measurement of filtration rate, urine osmolality and the free water clearance. When the observations are corrected to comparable filtration rates, the patients with nephrogenic diabetes insipidus are found to have the same relationship between urine flow, urine concentration and total solute excretion as the normal subject whose water intake has been sufficiently increased to inhibit release of endogenous ADH [*29b*]. In one patient exogenous vasopressin had an equivocal chloruretic action [*24*]; the problem warrants clarification to determine the extent to which the chloruretic and antidiuretic actions of ADH may be separated.

Recent interest in the mechanism of urinary concentration and dilution has focused attention on the architectural arrangement of the nephron. A single microdissection study of a patient with nephrogenic diabetes insipidus was reported by McDonald [*28*] and showed no abnormality in the distal tubule or the loop of Henle; routine histological studies have been essentially negative [*25,30,31*], and the absence of vacuolization has helped exclude potassium deficiency as an etiological factor. Varying degrees of hydronephrosis are common and are presumably the result of back pressure from the distended bladder; this may be an important factor in initiating renal damage during the course of the disease.

The early studies of the family reported by Williams and Henry [*27*] indicated that the trait was limited to males and was a sex-linked recessive transmitted by females. Subsequently, the complete syndrome has been reported in females [*24*]; in addition, a "carrier state" for heterozygous females has been postulated, based on the finding that the mothers and grandmothers of affected children display an impaired ability to concentrate the urine [*32*]. This finding should be studied further with a complete appraisal of renal function, particularly since an intermediate response of the kidney to presumably maximal amounts of ADH may afford an unusual opportunity to analyze the action of ADH.

Renal Tubular Acidosis. This syndrome results from a primary defect in the mechanisms by which the renal tubule normally contributes to the acidification of the urine. It was first reported in children by Butler et al. [*33*] and the essential physiological mechanisms were subsequently clarified in balance studies by Albright and his group [*34*]. The cardinal features are the presence of a metabolic acidosis with hyperchloremia, inability to form a sufficiently acid urine, and the absence of significant glomerular insufficiency. Secondary disturbances in calcium and potassium metabolism are often severe and may be the basis of the presenting symptoms.

The metabolic acidosis of uremic renal insufficiency is characterized mainly by an increase in the plasma concentrations of inorganic phosphate and sulfate. These ions are excreted by glomerular filtration and selective tubular reabsorption, and are produced in amounts approximately proportiontal to protein catabolism. Hence, if there is a marked reduction in glomerular function, the plasma concentration of these anions must rise (in a manner quite similar to that of urea) until that point is reached at which the plasma level is sufficiently high that the amount filtered in a reduced volume of glomerular filtrate is adequate to keep excretion equal to production. If this rise in plasma concentration were accompanied by a proportional increase in fixed cation, i.e., sodium, there would be no significant change in pH. However, other mechanisms tend to keep total osmolality constant; serum sodium concentration therefore does not increase, and the rise in fixed anions displaces anions of weaker acids (i.e., bicarbonate); the resultant depression of bicarbonate is indicative of a metabolic acidosis.

The pathogenesis of renal tubular acidosis is fundamentally different in that it involves the retention of a different species of fixed anion, and results from tubular rather than glomerular insufficiency. Serum phosphate and sulfate are not elevated. As a result of the tubular defect the ability to acidify the urine is impaired (*v. seq.*), resulting in excretion of a disproportionately large amount of bicarbonate. This, of necessity, must be accompanied by reabsorption of a relative excess of other extracellular anions,

notably chloride. When appropriate consideration is given to eventual osmotic adjustments and to total electrolyte balance, it is apparent that the tubular defect must become manifest as a change in extracellular composition with hyperchloremia and metabolic acidosis.

It was formerly believed that bicarbonate reabsorption and urinary acidification occurred as two separate processes, one in the proximal and one in the distal tubule. This concept had evolved from a variety of considerations of which the most important were (1) the demonstration that the pH decreased in the distal segment of the amphibian tubule [*35*]; (2) the observation that sulfanilamide, a carbonic anhydrase inhibitor, blocked the reabsorption of approximately 20 per cent of the filtered bicarbonate [*36*]; (3) the coincidental calculation that approximately 20 per cent of solute and water reabsorption could be attributed to the distal segment [*15*]; and (4) certain observations relating to pCO_2 of the urine [*37*]. It was thus proposed that approximately 80 per cent of the filtered bicarbonate was reabsorbed in the proximal tubule by a mechanism not dependent on carbonic anhydrase and that reabsorption of the residual bicarbonate, as well as the actual acidification of the urine, was a distal function dependent on carbonic anhydrase. It was proposed by Latner and Burnard [*38*] that the defect in renal tubular acidosis was in the proximal reabsorption of bicarbonate.

Largely as the result of the introduction of more potent inhibitors of carbonic anhydrase, it is now believed that the acidification processes throughout the tubule are qualitatively similar [*39*]. For operational purposes these may be described as the exchange of sodium ions from the tubular urine for hydrogen ions from the tubular cell [*37*]. The immediate effect of this exchange depends almost completely on the composition of the tubular urine at the exchange site. If bicarbonate is present, the addition of hydrogen ions leads to eventual bicarbonate reabsorption; if bicarbonate has been largely absorbed and buffer anion (such as phosphate) is available, acidification leads to an increase in titratable acid; and, if ammonia is being synthesized by the tubular cells, it diffuses more readily into an acid urine, thus tending to neutralize urinary acidity by the formation of ammonium ion.

Conflicting interpretations have resulted from the quantitative measurements of the acidification defect in renal tubular acidosis. Albright et al. [*34*] demonstrated that the urinary pH was inappropriately high and that the tubule appeared to be unable to respond to an acid load by a normal increase in ammonia and titratable acid excretion and by a fall in urinary pH. Latner and Burnard [*38*], however, were able to show in studies of children that urinary pH was not rigidly fixed but that it could be significantly decreased when the loading anion was phosphate rather than chloride. They interpreted their findings to indicate a limitation in the amount of bicarbonate reabsorbed proximally and suggested that this process was somehow facilitated by the presence of phosphate. This concept has been reexamined by Reynolds and Martin [*40*]. They have emphasized that infusions of sodium bicarbonate increase the rate of bicarbonate reabsorption (calculated as mEq. reabsorbed per 100 ml. of glomerular filtrate) without the necessity of administering phosphate. This phenomenon had been observed in earlier clearance experiments [*41*] and is also implicit in many balance studies.

These apparently conflicting observations may be resolved if due consideration is given to the multiple parameters of urinary acidification. Accepting the thesis that the proximal and distal mechanisms are qualitatively similar and that they both involve the cationic exchange of tubular sodium for cellular hydrogen ions, the acidification mechanism may be evaluated in terms of two functions, the total amount of hydrogen ion added to the urine and the gradient of hydrogen ion against which this secretion must occur. The studies by Pitts and his group [*42*] of normal subjects clearly indicated that the tubular secretion of hydrogen ion leading to the formation of titratable acid was primarily determined by the pKa of the buffer within the tubular urine. If this was very low, the secretory process rapidly lowered the pH of the urine and the total amount of titratable acid was relatively small. If the pKa was higher, for example around 6.7 in the case of phosphate, then the secretion of hydrogen ions had only a relatively small effect on urinary pH and the total titratable acid was large. The same type of analysis may be extended to bicarbonate reabsorption. By definition, hydrogen ion secretion leading to the formation of titratable acidity has to occur against a hydrogen ion gradient. However, in the case of bicarbonate reabsorption the same secretory process operates against a negligibly small

gradient and the magnitude of the secretory process is determined mainly by the amount of bicarbonate available in the glomerular filtrate.

Normally the minimal pH of the urine is about 4.5. Hence tubular secretion of hydrogen ions must cease when a gradient represented by this pH has been achieved; this occurs at a pH at which urinary bicarbonate concentration is virtually nil. However, in renal tubular acidosis the limiting hydrogen ion gradient occurs at a higher pH and the voided urine therefore contains little titratable acid and significant concentrations of bicarbonate. This eventually leads to metabolic acidosis with a decrease in plasma bicarbonate; the amount of bicarbonate both filtered and reabsorbed must therefore necessarily be reduced. However, the tubule is capable of secreting increased quantities of hydrogen ion if conditions permit a minimal gradient to be maintained. The infusion of sodium bicarbonate results in increased hydrogen ion secretion as measured by the amount of bicarbonate reabsorbed; likewise, the infusion of neutral phosphate increases hydrogen secretion as measured by a tenfold rise in titratable acidity with a negligible change in pH [*40*]. For reasons that remain obscure, increased phosphaturia is normally associated with a decrease in urinary pH independent of alterations in extracellular acid-base balance [*42*]. However, in patients with tubular acidosis the acidity of the urine increases less than in normal subjects, and in some patients not at all [*38,40*].

It is difficult to ascertain whether the defect in excretion of ammonium ion results from a failure of synthesis or from a decreased rate of diffusion secondary to diminished acidification of the urine. Estimations of renal glutaminase have not been reported. However, the importance of the over-all failure of ammonia excretion is clearly illustrated by the response to equivalent loads of acid as either ammonium chloride or acid phosphate [*40*]. Ammonium chloride produced progressive acidosis with little change in urinary excretion of ammonium ion. Since chloride cannot act as a buffer anion, the only compensatory mechanism available to the kidney is to increase excretion of ammonium ion, but this failed because of one or both of the factors previously mentioned. However, the acid phosphate load was readily handled by a fourfold rise in titratable acid which paralleled the increase in phosphate excretion.

Since both hyperchloremic acidosis and decreased acidity of the urine are characteristically produced by inhibitors of carbonic anhydrase, it has been postulated that the renal defect in tubular acidosis is a deficiency of this enzyme. At least four patients [*43,44*] have received acetazoleamide, which produced an increase in bicarbonate excretion typical of carbonic anhydrase inhibition. This strongly suggests that in the naturally occurring disease a defect of this enzyme cannot be implicated as the factor limiting the acidification mechanism.

The diminished ability of the tubule to exchange sodium for hydrogen would result in a simple increase in sodium excretion if sodium were not partially "replaced" by other cations. Balance studies clearly show increased calcium and potassium losses. Although these tend to minimize the loss of sodium, some depletion may occur [*41*], but not of a severe degree. The tubular mechanisms regulating calcium excretion have not been elucidated. The potassium loss may probably be attributed to renal tubular secretion, i.e., sodium-potassium exchange [*45*], possibly mediated by increased secretion of adrenal cortical hormones [*46*]. In contrast to many other conditions with potassium depletion, the urinary excretion of potassium in tubular acidosis tends to fall rather than to rise during treatment with sodium bicarbonate, possibly as the result of restoration of extracellular electrolytes towards normal and the consequent diminished stimulus to potassium secretion. Other renal functions remain unimpaired except in the few patients in whom glomerular insufficiency develops, probably as a result of nephrocalcinosis and repeated bouts of pyelonephritis. The capacity to secrete PAH and reabsorb glucose may be slightly reduced [*45,47*]. Renal glycosuria and amino aciduria do not occur [*7*]. Water reabsorption is partially impaired and may result from both potassium depletion and the tubular lesions associated with the nephrocalcinosis. Although the response to vasopressin is usually poor, definitive studies have not been reported.

The etiology remains obscure. The suggestion that injudicious sulfonamide administration might produce irreversible carbonic anhydrase inhibition has not been borne out. Many patients give no history of this type of medication, and the recent widespread use of acetazoleamide has certainly not produced irreversible enzyme inhibition. The possibility that the tubule is damaged by a primary pyelonephritis would

appear untenable at least in children, since in them the defect is readily reversible and is unrelated to signs of active infection. Multiple cases have now been reported in at least four separate families, and it is possible that the syndrome represents a hereditary trait [*48–51*].

Morphological studies have failed to reveal lesions which can be correlated with the primary functional disturbance. Deposits of calcium are frequently seen, particularly in the peritubular spaces near the pyramids. The sequence of events is thought to be failure of acidification, systemic acidosis with mobilization of skeletal calcium, hypercalcuria, and then the urinary precipitation of calcium within the distal tubular system. Precipitation of calcium is facilitated by the alkaline urine and possibly by the absence of solubilizing anions such as citrate [*52*]. Vacuolization of the proximal tubule has also been noted [*53*] and is probably the result of potassium depletion rather than the sign of a primary lesion.

The disorder is frequently encountered in young children [*54,55*], and appears to be physiologically indistinguishable from the adult type. However, most of the children recover spontaneously, but the factors involved are unknown. Return of normal tubular function is rare in adults, although it has been reported [*44*]. Treatment of the electrolyte disorder results in a remarkable disappearance of symptoms. Vitamin D and increased calcium intake are usually needed only until the osteomalacia is healed. Alkalinizing agents readily correct the acidosis and maintenance doses are required by most adults. This regimen, of course, does not modify the underlying defect. Although renal calculi may continue to be formed, follow-up studies for at least ten years indicate that alkali therapy does not induce further renal damage and that the course may be quite benign [*56*].

Salt Losing Nephritis. Since this term has been widely employed, it is included here for the sake of completeness. In patients with advanced renal disease and nitrogen retention, an excessively large fraction of the filtered sodium and chloride is excreted in the urine [*57,58*]. In some patients the failure of sodium reabsorption may become of paramount importance, and severe sodium depletion may result However, there is no evidence that the inability to reabsorb sodium chloride is a primary defect or that it can occur as an isolated tubular dysfunction. An apparent exception to the latter statement is the physiological abnormality of Addison's disease, but in this instance the primary defect is clearly adrenal and not renal in origin.

Potassium Losing Nephritis. Had this review been written before the publication of Conn's paper in 1955 [*59*] it is probable that "potassium losing nephritis" would have been included as an example of a primary tubular defect. Subsequent studies have shown that most cases, although perhaps not all, are secondary to aldosterone-producing tumors of the adrenal cortex. However, excessive urinary potassium excretion undoubtedly occurs in association with several tubular syndromes, especially renal tubular acidosis and the multiple defects of the Fanconi type. Currently, the only clear-cut evidence for an isolated renal abnormality involving inappropriate potassium excretion resides in the observations that severe depletion can occur in the absence of other demonstrable causes [*60–63*]. This general problem is under active study in a number of laboratories, and significant clarification can be anticipated.

Renal Hypophosphatemia. In areas in which the dietary intake of vitamin D is adequate, this syndrome represents the most common cause of rickets in childhood. Physiologically, it is characterized by the urinary excretion of normal amounts of phosphate despite reduction in the plasma concentration. The phosphate clearance is therefore abnormally high. This syndrome is commonly designated vitamin D resistant rickets or renal hyperphosphaturia, but the present nomenclature seems more appropriate since it accurately denotes the functional disturbance. Therapeutically, the most distinctive feature is the failure to respond to the usual dose of vitamin D, but a generally satisfactory response to extremely large doses.

Other renal functions are within normal limits; there is no glycosuria or aminoaciduria. The balance studies of Robertson et al. [*64*], and of Dent [*7*] clearly defined the role of the tubular defect in the pathogenesis of the skeletal lesions. While in a normal subject urinary phosphate excretion decreases in the presence of marked hypophosphatemia, in these patients there is little fluctuation in renal phosphate excretion when the plasma phosphate concentration varies from the spontaneously low levels to the more normal range obtained after massive vitamin D therapy. Presumably, the tubular defect resides in failure of the phosphate reabsorptive mechanism to respond to the usual

amounts of vitamin D. Harrison [*52*] has noted, however, that healing of rickets begins before the hypophosphatemia is corrected and therefore believes that hypophosphatemia is not the sole factor in the pathogenesis of the rickets.

Careful genetic analysis reveals that the defect is transmitted as a sex-linked dominant [*65*]. The full syndrome is encountered in male children of matings in which one or the other parent is hypophosphatemic. When the trait is transmitted by the mother, her hypophosphatemia is usually asymptomatic, suggesting that a normal allele ameliorates the manifestations of the underlying disorder. Of considerable interest is the finding that the Tm for phosphate is reduced to about one-half normal values in both the hemizygote males and the heterozygous females. As in other reabsorptive defects, urinary excretion occurs at loads considerably less than those required to saturate the Tm. Phosphate titration curves might be of considerable interest.

McCance [*66*], and Dent and Harris [*67*] have suggested that a related disorder occurs in young adults which is sufficiently distinct to warrant separate consideration. In brief, osteomalacia appears after an asymptomatic childhood and may be associated with severe muscular disability. Slight systemic acidosis has been noted, but there is no apparent defect in urinary acidification. Urinary calcium excretion is less than normal; it is not increased as in tubular acidosis. The tubular defect involves failure to reabsorb phosphate in the face of extremely low plasma levels. Another abnormality has been mentioned but its significance is not clear: In four patients, Dent's group found a marked increase in glycine excretion but no disturbance in the urinary pattern of other amino acids [*67*].*

Abnormalities of Urate Reabsorption. The normal tubular mechanisms of urate transport are not sufficiently understood to permit a precise definition of abnormal function. Studies with acutely elevated blood levels have indicated a reabsorptive Tm [*16*]. In gouty subjects with chronic hyperuricacidemia, the unpredictable relationship of the plasma level to the amount of urate excreted has prompted Gutman and Yü [*68*] to suggest that the tubular mechanism may be one of both reabsorption and secretion. While conclusive evidence has not been obtained, the paradoxical response to uricosuric agents suggests such a possibility. In small dosage, salicylate and phenylbutazone decrease urate excretion, while in larger dosage these drugs become uricosuric. In addition, pyrazinamide markedly decreases urate excretion without affecting the amount filtered.

Of considerable interest is the isolated case report of Praetorius and Kirk [*69*] who studied an asymptomatic young man who was found to have a plasma urate concentration about one-tenth the normal value, but who excreted a normal amount of urate per day. Renal function studies revealed that the filtration rate was normal and that the urate/inulin clearance ratio was approximately 1.50 compared to a normal value of about 0.10. At first glance the data suggest a primary tubular defect with an excessively high urate clearance due to tubular secretion. However, evaluation of the role of the kidney is complicated by the finding that the plasma concentration of the oxypurine precursors of urate was excessively high, suggesting a general metabolic defect as the primary disturbance.

The urate clearance is elevated in the adult Fanconi syndrome, whether idiopathic or secondary to Wilson's disease [*70*]. In one well studied subject the urate clearance was identical with the filtration rate and did not change following the administration of probenecid [*71*]. This strongly suggests a defect in urate reabsorption, rather than secretion.

Simple Xanthinuria. Dent and Philpot [*72*] have described a female child who excreted excessive amounts of xanthine. The authors emphasized that a pre-renal defect in xanthine metabolism had not been excluded with complete certainty, but their data disclosed low levels of both uric acid and xanthine in the plasma, strongly suggesting that the increased xanthinuria was renal in origin. Further studies indicated the absence of generalized aminoaciduria, a normal calcium metabolism, and no evidence of glomerular insufficiency. The patient was in good general health except for the passage of a renal stone which was found to be composed entirely of xanthine.

In this patient the daily urinary excretion of xanthine was approximately eight times greater than that of a control subject. Examination of the stone and of the urine demonstrated the absence of uric acid, which was also virtually undetectable on chromatographic analysis of the plasma. This combination of findings is consistent with an over-all deficiency in the conversion

* Glycine decreases phosphate Tm in the dog [*122*]. The finding is of theoretical interest but its bearing on this clinical syndrome is unknown.

of xanthine to uric acid and raises the possibility that the primary defect may be an excessively high renal clearance of xanthine. This would maintain a low concentration of xanthine in plasma and possibly in other body fluids. On this basis the failure of the enzymatic oxidation of xanthine to uric acid would be attributable to low concentrations of substrate rather than to the absence of specific enzymes. Careful analysis of three generations revealed no additional cases and no history of consanguinity. No histological studies have been performed.

Generalized Aminoacidurias. Largely as a result of the introduction of chromatographic analysis for urinary amino acids it has become evident that renal aminoaciduria may be encountered in a wide variety of conditions. A relatively small number of disorders involving distinct and separate amino acids have been recognized and will be individually described. However, the most commonly observed pattern of aminoaciduria is so generalized, and in some instances so variable, that it would at present be unrealistic to describe each example as a specific tubular dysfunction.* Indeed, as far as specificity is concerned, generalized aminoaciduria may some day have the same significance for tubular dysfunction that albuminuria now has for the more common types of renal disease. In addition, aminoaciduria may prove a more sensitive index of injury than other tests of tubular function.

Generalized renal aminoaciduria occurs in: infantile cystinosis [*73,74*], adult type of Fanconi syndrome [*75–77*], heavy metal poisoning [*78,79*], Wilson's disease [*80*], multiple myeloma [*81*], galactosemia [*82–85*], scurvy [*86*], rickets due to lack of vitamin D [*87*], lysol poisoning [*88*] and progeria [*89*]. Additional causes may certainly be anticipated. The specific renal aminoacidurias include: essential cystinuria, glycinuria, Hartnup's disease and β-amino*iso*butyricacidura.

Some of the general aminoacidurias are discussed under "Multiple Tubular Defects"; little can be said about many of the others from the point of view of tubular function. However, galactosemia is of considerable interest as an example of an inborn error in which the renal disturbance is quite clearly a complication of the primary abnormality. The disease results from an inability to metabolize galactose, leading to high blood levels and increased urinary excretion. The enzymatic defect has been identified in erythrocytes and in the liver. (See Holzel et al. for review [*85*].) When affected subjects are fed a galactose-free diet the signs of abnormal carbohydrate metabolism disappear. In several studies of the course of the aminoaciduria it has been found that the amino acid excretion is normal on a galactose-free intake, that it appears approximately one week after the galactose intake has been increased, and that it subsequently subsides several days after a diet low in galactose has been reinstituted. This sequence of events argues strongly against the thesis that galactose and amino acids are reabsorbed by a common mechanism and that the aminoaciduria results from a competitive type of inhibition. It has been proposed that high levels of galactose in body fluids have a deleterious effect which underlies the damage that occurs in the liver, cornea, central nervous system and kidney. Holzel et al. have reviewed the problem and believe that a metabolic product of galactose, rather than galactose itself, is the toxic agent. Tubular mechanisms other than these involving amino acid transport have not been systematically investigated.

Essential Cystinuria. Largely as a result of the studies of Dent and Harris and their colleagues, the genetic and physiological basis of this syndrome has become clearly defined. The condition is benign except for the sequelae of renal calculi. The renal defect is limited to the reabsorption of cystine, lysine, arginine and ornithine. Measurements of filtration rate are within normal limits [*90,91*], there is no glycosuria, and no apparent disturbance in mineral or water balance. Studies of cystinuria in canines reveal a similar type of renal defect in that both lysine and cystine excretion are definitely increased [*92*].

Normally the renal clearance of cystine is about 4 ml. per minute [*90*]; thus over 95 per cent of the cystine filtered at the glomerulus is reabsorbed by the tubules. In cystinuric subjects virtually no cystine is reabsorbed, leading to a rise in urinary excretion and a fall in plasma levels to about one-half the normal values. In contrast to other tubular syndromes such as renal glycosuria, in the cystinuric subject reabsorption does not increase in response to a rise in filtered load, indicating that the defect is an absolute one and that it is unrelated to the population type of defect. However, it would be of interest to determine titration curves of glucose or PAH transport in these patients.

* The most commonly observed patterns of aminoaciduria are documented elsewhere [*77,86*].

The limitation of the disturbance to four specific amino acids has suggested that these are normally reabsorbed by pathways which have certain features in common. When lysine was infused into a normal subject there was a transient rise in the excretion of cystine, ornithine and arginine; this was interpreted as showing that the increased concentration of lysine had displaced the other amino acids from the transport mechanism [*93*]. However, in the cystinuric subject infusions of lysine had no effect on the already elevated rate of excretion of these amino acids, presumably because the natural defect in reabsorption was so complete that it could not be depressed further. In neither instance did lysine loads result in a general aminoaciduria.

Although most attention has been focused on cystine because of the relationship of its insolubility to calculus formation, the renal abnormality involves other amino acids to a significant degree.* Quantitative studies have shown average excretion rates to be approximately 1.8 gm. of lysine, 0.8 gm. each of cystine and arginine, and 0.4 gm. of ornithine per day [*94*]. The detailed measurements of lysine excretion reveal a defect similar to that of cystine [*91*]. Thus about 99 per cent of the filtered lysine is reabsorbed in normal subjects, but only about 45 per cent in the cystinuric subjects. As a consequence endogenous excretion is high, and plasma levels are depressed. Although small amounts of lysine may be normally absorbed by these patients, this reabsorptive capacity is limited since a moderate increase in lysine load results in a clearance essentially equal to the glomerular filtration rate. Preliminary studies suggest a similar limitation of arginine reabsorption [*91*].

The detailed family studies which have been summarized by Dent and Harris [*10*] have indicated that there may be two different types of essential cystinuria, but that both are clearly separate from the disturbance in cystine metabolism that is sometimes associated with multiple tubular defects (Fanconi syndrome). In one group of cystinuric families the abnormality is transmitted as a typical mendelian recessive which appears in the affected homozygotes as a disturbance in the reabsorption of the four amino acids. In the second group, which has been termed "incompletely recessive cystinuria," three classes of individuals have been detected—those with marked increase in the excretion of all four amino acids, an intermediate group with cystinuria and lysinuria but no increase in ornithine or arginine, and those with no phenotypic defect. On this basis it has been postulated that ornithine and arginine have a greater affinity for the transport mechanism than do lysine or cystine.

Doolan et al. [*91*] have reported a novel approach to the analysis of renal transport by determining the rate of glycocyamine excretion following the administration of both arginine and glycine. This procedure was based on the previous demonstration that the transamination reaction between arginine and glycine occurs only in the kidney, and that a decrease in the excretion of the product of the reaction, i.e., glycocyamine, might occur in a disorder characterized by unusual impermeability of the kidney cells to one or more of the reactants. In three patients glycocyamine excretion was within normal limits, suggesting that adequate arginine entered the cells of the kidney either from the luminal or peritubular surface. While the results are negative, the use of an organ-specific synthetic reaction constitutes a new type of renal function test which may be of some promise.

Simple Glycinuria. The recent report by de Vries et al. [*95*] on hereditary glycinuria adds a new syndrome involving a single isolated defect in tubular transport. Four female subjects in three generations of one family were found to excrete excessive amounts of glycine; in three, this was associated with nephrolithiasis. Otherwise, they were asymptomatic, and growth and development had been normal. The specificity of the renal lesion was clearly indicated by detailed studies. The tubular transport of glucose, phosphate and calcium were normal even when the filtered load was increased. Blood urea nitrogen and uric acid concentration were within normal limits. Although the acidification mechanism was not tested directly this was presumably unimpaired as indicated by normal plasma concentrations for bicarbonate, chloride and potassium. Urine specific gravity suggested a normal response to ADH.

A renal calculus was removed from one patient

* Dent [*76*] has observed that the blotched genet, a wild cat native to Kenya, normally excretes cystine in concentrations at least twice those obtained by cystinuric subjects, but that no instances of cystine calculi have been reported. The significance of the observations awaits further study.

and was found to be almost entirely composed of calcium oxalate; approximately 0.5 per cent of the dry weight of the stone was glycine. This raised the possibility that the excessive glycine within the tubular urine might have been metabolized to the much less soluble oxalate. However, the excretion of oxalic acid was within normal limits.

Glycine was the only amino acid found to be excreted in excessive amounts; that this was renal in origin was indicated by the normal chromatographic intensity of the serum glycine spot. Genetic studies suggested a dominant pattern of inheritance. Histological analysis of the kidneys is not available. Since glycine is a precursor of glycocyamine, it would be of interest to ascertain whether or not these patients can synthesize this compound (see discussion of essential cystinuria).

Hartnup's Disease. This is an extraordinarily complex clinical syndrome best described by the title of the definitive paper "Hereditary Pellagra-like Skin Rash with Temporary Cerebellar Ataxia, Constant Renal Aminoaciduria, and Other Bizarre Biochemical Features" [*96*]. Two siblings were initially observed. Further examination revealed that the parents were first cousins and that four of the eight children had a strikingly abnormal amino acid pattern in the urine, although clinical symptoms were present in only two. On further search of the literature the authors found two suspected cases which were subsequently definitely diagnosed by chromatographic examination of the urine. No histological observations have been reported.

Renal function was characterized by the normal excretion of urea, the absence of proteinuria and glycosuria, normal plasma electrolyte values with a normal response to an acid load, and the absence of any disturbance in calcium or phosphorus metabolism. The urine of each patient contained an unusual pattern of indoles, but there are insufficient data to determine whether or not this was of renal origin. Plasma concentrations of amino acids were normal, measured either as α-amino nitrogen or as individual amino acids, and indicated that the amino aciduria was renal in nature. The most striking finding was the pattern of the individual amino acids that were excreted, this being so unusual that the authors suggested that it be designated as the "Hartnup amino acid pattern." Although several amino acids were involved, the absence of any increase in proline excretion constituted the single most important characteristic by which this pattern could be distinguished from the more common aminoacidurias of Fanconi's syndrome or Wilson's disease.

The authors speculated that the entire syndrome might result from a biochemical abnormality related to nicotinic acid metabolism. While the exact pathogenesis remains in doubt, the renal defect is of exceptional interest since its causal relationship to the rest of the syndrome has as yet not been clarified.

β-Aminoisobutyric Aciduria. In extensive chromatographic studies of human urine it was found that about 5 per cent of subjects excreted a large excess of an amino acid which was eventually identified as β-amino*iso*butyric acid [*97*]. These subjects are asymptomatic and suffer from no overt renal disorder. Detailed quantitative studies in one subject indicated a renal clearance of 85 ml./minute, compared to normal values ranging from 0 to 4 ml./minute [*77*]. The plasma levels are not abnormal and the findings clearly revealed a diminished rate of tubular reabsorption. Similar urinary patterns were found in high incidence among relatives. While clinically unimportant, the finding has potential heuristic value.

Multiple Tubular Defects. The syndromes that are usually associated with the names of Lignac, Debré, de Toni and Fanconi have been extensively studied and many well documented case reports are now available. The recent clarification of some of the confusing problems has resulted largely from the realization that there are multiple etiological factors to which the tubule can respond in only a limited number of ways. The disturbances that are most frequently encountered together include renal glycosuria, renal aminoaciduria and renal hypophosphatemia, the latter leading either to rickets or osteomalacia, depending on the age of the patient.* Since these defects are generally attributed to proximal tubular defects, it is worthy of note that sodium chloride reabsorption remains essentially unimpaired and that severe salt depletion does not occur. In some subjects the disturbance may be more generalized and involves to a variable degree the renal regulation of water, potassium, hydrogen ion and urate excretion.

From general etiological considerations, at

* Fanconi's name is now generally used as an eponym for this pattern of renal failure.

least five disease entities may now be recognized: (1) the childhood type with cystinosis, (2) the adult type without cystinosis, (3) heavy metal poisoning, especially lead, (4) Wilson's disease, and (5) multiple myeloma. Further additions to this list may certainly be anticipated.*

The infantile type is associated with an abnormality of cystine metabolism leading to the deposition of cystine crystals in many organs, particularly the reticuloendothelial system, cornea, bone marrow and, in some instances, the kidney. (For review see [*98*].) The cystinosis itself denotes a poor prognosis, and eventually may lead to glomerular insufficiency with uremia. Its pathogenesis remains obscure. The plasma cystine level is not elevated and cystine excretion in the urine may or may not be increased. It has been proposed that the cystinosis is either independent of the renal defect or occurs prior to renal involvement. Serial observations on newborn children, in whom the disease was anticipated because of the family history, showed that urinary function was normal until the fifth month of life when aminoaciduria, glycosuria, and cystine crystals in the cornea all appeared at about the same time [*99*]. Many studies have indicated a familial incidence but cases from more than one generation in a family have not been described. A simple mendelian recessive is indicated by the study of Bickel et al. [*98*]. It should be emphasized that this syndrome appears genetically unrelated to the adult type of Fanconi's syndrome or to essential cystinuria [*10*].

The adult cases are not associated with cystinosis but the manifestations of renal dysfunction are otherwise similar. The disease usually begins with symptoms either of osteomalacia or of potassium depletion. One adult had had rickets as a child [*100*]. Some children with the renal disturbance, but without demonstrable cystinosis, may indeed be examples of the "adult" type. Dent and Harris [*10*] concluded that the adult trait was a simple mendelian recessive. Of considerable interest in their study was the discovery of three siblings who were asymptomatic but who had aminoaciduria, glycosuria and hypophosphatemia; presumably their renal disturbance was not sufficient to produce a significant loss of calcium or potassium, hence the absence of symptoms.

Scattered observations have long suggested that heavy metal poisoning may be associated with the development of one or more features of Fanconi's syndrome. Rickets was reported in cadmium workers by Nicaud et al. [*101*], and aminoaciduria occurs in industrial workers exposed to lead, mercury, uranium and cadmium [*79*]. Caffey reported a child with active rickets, glycosuria and lead poisoning, but other biochemical studies were not performed [*102*]. In another study lead poisoning was associated with aminoaciduria and glycosuria but apparently not with overt rickets [*78*]. Recently, Chisolm et al. [*103*] have studied a case of lead poisoning with aminoaciduria, glycosuria, hypophosphatemia and rickets; at least eleven additional cases have now been observed by them in which acute lead poisoning in children was associated with similar renal defects. Following appropriate treatment of the lead poisoning, the signs of disturbed renal function disappeared. Experimentally, subacute heavy metal poisoning, particularly with uranium salts, is associated with proximal tubular lesions [*104*].

Wilson's disease (hepatolenticular degeneration) has recently been characterized as an inborn error in the metabolism and distribution of copper, leading primarily to disturbances of hepatic and central nervous system function, but also to kidney involvement with the Fanconi pattern of tubular insufficiency. Advanced osteomalacia is occasionally observed [*105a*], apparently as a result of hypophosphatemia due to excessive excretion of phosphate in the urine [*105b*]. The glycosuria has been reported to be associated with a reduced glucose Tm. Tissue analyses reveal a striking increase in the copper content of the kidney [*106*]. In one report, asymptomatic siblings were found to have aminoaciduria without other demonstrable evidence of Wilson's disease [*107*].

A similar syndrome has been noted in two adult patients with multiple myeloma [*81*] and in a child with glycogen storage disease [*108*]. In both instances the findings suggest that the renal disturbance was secondary to the underlying disorder.

Is it possible, then, to formulate a concept of the pathogenesis of Fanconi's syndrome? Granting that multiple etiological factors are involved, certain features stand out: first, the familial incidence; second, the frequency with which heavy metal poisoning is encountered; third, the period immediately after birth when the infantile cases show normal renal function; and

* Experimentally, the syndrome may be produced by the administration of maleic acid [*52*].

fourth, the apparent reversibility in some patients after massive vitamin D therapy [*99*]. All these factors suggest that the renal defect is not an absolute one, such as occurs in essential cystinuria, and that the multiple disturbances in tubular transport may be responses to underlying tubular injury. If so, then the simultaneous failure of glucose, phosphate and amino acid reabsorption cannot be regarded as evidence that these substances are reabsorbed by mechanisms which have specific functional attributes in common. It has been suggested that in the infantile cases cystine deposits within the cells of the tubule may disrupt normal function. However, it is also tempting to speculate that the underlying abnormality may be a failure in the metabolism of heavy metals which are normally present in trace amounts or that the high familial incidence limited to single sibships may be related to environmental factors involving undue exposure to heavy metals. Renal aminoaciduria subsides when vitamin D is administered for treatment of rickets of dietary origin [*87*] and, in at least one well studied case of cystinosis, both glycosuria and aminoaciduria disappeared following administration of vitamin D [*99*]. Freudenberg [*109*] had suggested that hypophosphatemia might effect the normal intracellular balance of organic phosphates involved in active transport mechanisms. However, simple renal hypophosphatemia (vitamin D resistant rickets) is not associated with other tubular dysfunctions. An additional hypothesis warrants further study, namely that vitamin D affects not only the metabolism and distribution of phosphorus and calcium but also, even if indirectly, that of other divalent cations, including heavy metals.

Although possibly tangential, it is appropriate to point out that the renal excretion of at least one heavy metal (i.e., mercury) involves tubular secretion, and that the excretory product is the mercuric-cysteine complex. Secretion has been thoroughly documented for organic mercurials [*110*], but probably holds true for inorganic mercury as well [*111*].* Whether or not other heavy metals are secreted by the tubule in a similar manner is not known, but their nephrotoxicity is well established.

On the basis of morphological evidence, the pathogenesis of Fanconi's syndrome still remains obscure. In a limited number of studies of the infantile type, cystine deposits have been identified within the cells of the tubule. In addition, there is both tubular and glomerular damage of a non-specific nature. Several authors [*112*] have described intensive vacuolization of the tubular cells, a finding which is suggestive of secondary potassium depletion rather than a primary tubular lesion. The most striking findings have been reported by Darmady and his group [*113*] who showed with the microdissection technic an area of marked atrophy in the most proximal position of the nephron, producing a narrow segment resembling a "swan's neck." From other considerations it is tempting to regard such a lesion as the expression of a genetic defect; however, there is no *a priori* reason why atrophy of this segment could not be the result of a more fundamental functional disturbance. The studies of Bickel [*99*] on the chronology of the renal lesion of the infantile type indicate that the defect in reabsorption is not present at birth; thus, if the swan's neck lesion has physiological implications, it would appear not to be congenital but rather to be acquired during the early months of postnatal development. Early reports showed the absence of phosphatase in the proximal tubule but little evidence has been forthcoming to support the notion that this enzyme is directly involved in the transport of the substances concerned [*75*].

Considerable uncertainty accompanies evaluation of the role that the renal defect plays in the pathogenesis of the total syndrome. Dent has doubted that the aminoaciduria is of itself of sufficient magnitude to cause an amino acid deficiency [*76*], although depletion of specific amino acids is not excluded. Similarly, glycosuria is not massive and can contribute but little to total caloric balance. Potassium depletion results from excessive renal excretion [*71,114*], and may be of considerable importance as a cause of the untoward reactions of acute hypokalemia which are encountered during glucose tolerance tests. Calcium and phosphate balances normally represent a delicate adjustment between intestinal absorption and renal excretion. The careful studies of Bickel et al. [*98*] indicated that negative balances were a consequence of decreased absorption rather than of increased urinary excretion, and Dent [*73*] has also reported the development of skeletal lesions before the appearance of hypophosphatemia. Although the initiating events may remain obscure, there

* In another paper in this symposium Pitts presents evidence localizing the secretion of organic mercurials to the proximal tubule.

can be little doubt that hypophosphatemia becomes perpetuated by the excessively high renal phosphate clearance [*71,114*]. Nephrocalcinosis has not been reported in Fanconi's syndrome, although one adult had renal calculi [*100*]. This contrasts with its high incidence in renal tubular acidosis and is of particular interest since some patients with the Fanconi type appear to have defective acidification mechanisms. Several factors may be involved. Hypercalcuria is less prominent in Fanconi's syndrome than in renal tubular acidosis, and urinary pH is frequently low even though acidification defects may be evident from other measurements. In addition, the high excretion rate of amino acids and possibly of other organic acids may serve to chelate calcium and thus to prevent its precipitation [*115*].

Only limited studies are available on the quantitative evaluation of tubular function. The glucose Tm has been reported either as normal [*100,116*] or as reduced [*71*], and a marked decrease in PAH Tm has also been noted [*71*]. Lathem et al. [*116*] found that amino acid reabsorption increased with amino acid loading and interpreted this as consistent with a "population defect." The tubular transport of glucose and phosphate can also be elevated by increasing the load [*71,114*]. The effect of lysine loads on cystine excretion resembles that seen in normal subjects rather than that observed in patients with essential cystinuria [*93*]. These several lines of evidence indicate that the transport mechanisms are not absolutely defective, in comparison to what occurs in essential cystinuria. However, one careful analysis of urate excretion indicated a complete failure of reabsorption [*71*]. It is probable that there is considerable individual variation in the degree of renal functional impairment.

Probably the majority of patients with Fanconi's syndrome have metabolic acidosis even in the absence of nitrogen retention, but the causes remain obscure. High plasma levels of organic acids have been reported; these have been based on indirect methods and have not been found by all observers [*112,114*]. However, hyperaminoacidemia is adequately ruled out. Some patients appear to have hyperchloremia. Although this suggests a disturbance similar to that of renal tubular acidosis, the pH of the urine is variable and in some subjects may fall as low as pH 5. In others there may be a relatively constant alkaline urine. Ammonia excretion varies enormously: it is either appropriate to the urinary pH [*114,117*], greatly elevated [*112*], or markedly reduced [*118*]. Sirota and Hamerman [*71*] infused phosphate into an acidotic patient and found only a slight increase in titratable acid excretion, but there was a simultaneous rise in urinary pH. However, the patient had diffuse renal impairment; similar studies on early and relatively uncomplicated cases are not available. The potassium clearance is often increased, and in one subject was significantly higher than the filtration rate [*71*], a finding consistent with the thesis that an augmentation in the rate of sodium-potassium exchange constitutes a partial compensation for the failure of acidification with its attendant loss of fixed cation. No studies have been reported with either organic mercurials or carbonic anhydrase inhibitors.

Atypical Multiple Defects. Several reports may be summarized for completeness, but without intent of establishing them as firm clinical entities.

Luder and Sheldon [*119*] reported the presence of renal glycosuria and renal aminoaciduria but without evidence of hypophosphatemia, rickets or osteomalacia. Studies on a pair of twins, their parents, and the paternal grandfather suggested an inherited trait. On the basis of routine clinical evidence, other renal functions appeared normal and the general course of the disorder was quite benign. A similar case has been observed by Harrison [*23*].

In 1952 Dent [7] reported four cases of glycosuria in association with a defect in phosphate reabsorption, but with no apparent disturbance in amino acid excretion. He questioned the validity of this entity as a distinct syndrome since a sibling of one patient had phosphaturia alone, without glycosuria. However, the disorder is worthy of note primarily because it is an apparent exception to the subsequent experience that aminoaciduria may be the most sensitive index of multiple tubular defects. Detailed renal function studies have not been reported.

Lowe and his associates [*120*] have studied several infants who presented some of the features of Fanconi's syndrome, including acidosis, rickets and glycosuria, but in whom the organic aciduria was not attributable to amino acids. The excretion of large quantities of non-amino organic acids is largely based on indirect evidence; in one patient pyruvate was conclusively identified [*121*] but accounted for only a small

fraction of the total organic acid excretion. A deficiency in ammonia production has also been described. The exact nature of the syndrome has not been established and the findings are insufficient to permit an appraisal of the role of the kidney in the pathogenesis of the total disorder.

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Seminar on Liver Disease

Pathogenesis and Management of Hepatic Coma*

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THE association of neuropsychiatric changes and liver disease has been known since antiquity. Hippocrates (460–370 B.C.) described a patient with hepatitis who "barked like a dog, could not be held and said things that could not be comprehended" and Frerichs [*1*], the father of modern hepatology, recognized noisy delirium and coma as complications of cirrhosis. The pathogenesis of these changes remained obscure until the present century. The development by Mann, Bollman and their group [*2*] of a successful technic for hepatectomy in animals and improvement in biochemical methods provided the stimulus to investigate biochemical changes in patients with hepatic coma. However, the hepatectomised dog becomes hypoglycaemic and has a low blood urea level, but patients in hepatic coma only rarely show these changes. Research had come virtually to an impasse until results obtained at the end of the last century by the Pavlov school [*3*] were reconsidered. This group showed that in dogs with an Eck fistula (portacaval anastomosis) meat intoxication developed and, from this beginning, the whole concept of ammonium toxicity in liver failure has been elaborated. The present review discusses the processes which culminate in the syndrome of hepatic coma in patients with liver disease. The problems presented by this condition cannot be regarded as solved but the advances made in the last ten years are well worthy of discussion.

THE CONCEPT OF PORTAL-SYSTEMIC ENCEPHALOPATHY

It is suggested that patients with liver disease, going into hepatic coma, are suffering from cerebral intoxication by intestinal contents which have not been metabolised by the liver. Certainly every patient presenting these features has a circulatory pathway through which portal blood may enter the systemic system and reach the brain without being metabolised by the liver [*4,5*].

In patients with poor hepatocellular function the shunt is through the liver itself. In the more chronic forms the portal blood bypasses the liver through large natural "collaterals." The portal-hepatic vein anastomoses developing around the nodules in a cirrhotic liver may also act as internal shunts. This picture of "intermittent stupor" complicates 15 to 20 per cent of surgical portacaval anastomoses [*6,7*]. It is analogous to the neuropsychiatric disturbances of the dog with an Eck fistula that is given meat.

The nature of the cerebral intoxicant(s) is speculative. A picture indistinguishable from impending hepatic coma can be induced in some patients with cirrhosis by the oral administration of a high protein diet, ammonium chloride or urea [*8–10*]; this suggests a nitrogenous nature. Similar toxic symptoms can be induced in certain patients with cirrhosis by methionine and these are prevented or retarded by chlortetracycline [*11*]. Neomycin given orally is particularly effective in the treatment of cirrhotic patients with protein intolerance [*12,13*]. The small intestinal bacterial flora is increased in patients with liver disease [*14*]. These facts all point to the intoxicant being produced by intestinal bacteria acting on the nitrogenous content of the intestine and especially on protein.

RELATION TO AMMONIUM TOXICITY

Monguio [*15*] demonstrated that blood ammonium levels were high in the dog with an Eck fistula suffering from meat intoxication. Van Caulaert and co-workers [*16*] and Kirk [*4*] related the elevations of blood ammonium in

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patients with cirrhosis to the diversion of portal blood into the systemic veins. Ammonium is present in high concentration in portal vein blood but is normally metabolised by the liver to urea [*17,18*]. It can be derived from bacterial action on nitrogenous material in the intestine

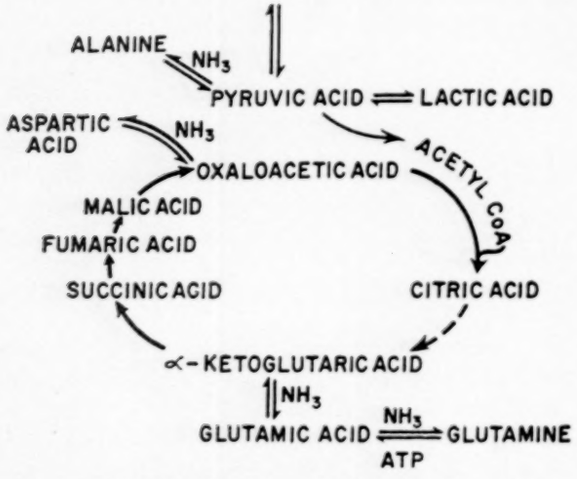


FIG. 1. The Krebs citric acid cycle.

[*19,20*]. Ammonium salts are toxic to some patients with cirrhosis when administered both orally and intravenously [*10,16,18,21*].

Elevations of blood ammonium levels in liver disease need not be related only to production from intestinal contents. The failing liver may also be unable to metabolise ammonium formed by the kidney and, in the terminal stages of hepatic coma, by the peripheral tissues and the brain [*22*].

Theoretically, ammonium intoxication could interfere with cerebral metabolism [*23*]. This could be by two mechanisms, glutamine synthesis and reductive amination of alpha ketoglutarate. (Fig. 1.) The combination of the increased ammonium in hepatic coma with glutamic acid to form glutamine is suggested by the increased amounts of glutamine in the cerebrospinal fluid [*24*].

Combination of ammonium with alpha ketoglutarate to form glutamic acid removes an important link in the Krebs citric acid cycle. The brain depends for most of its activity on aerobic glycolysis and on this cycle. The diminished oxygen consumption of the brain in hepatic coma [*25*] and the increased blood and cerebrospinal fluid pyruvic acid concentrations [*22,26–28*] would support this hypothesis.

Ammonium therefore fulfils all the criteria necessary for a substance responsible for hepatic coma. It is a cerebral intoxicant, a substance formed in the intestine from protein, and one that is normally metabolised by the liver. Ammonium poisoning has even been used synonymously with hepatic coma. There is, however, some evidence that this is too sweeping an

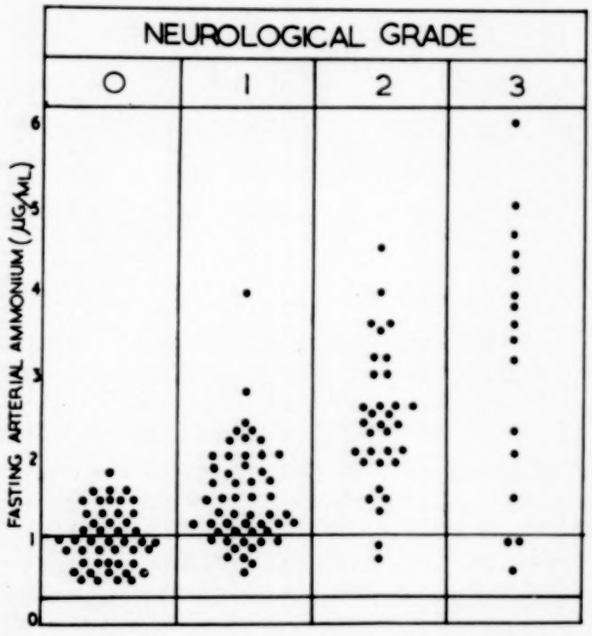


FIG. 2. The relation of the arterial ammonium level to the grade of consciousness in hepatic cirrhosis. *Grade 1:* minor disorder of consciousness and the motor system. *Grade 2:* gross disorder of consciousness with disorientation in time and space. *Grade 3:* coma. Mean normal arterial ammonium level is 0.68 μg./ml. The horizontal lines denote mean ± 2 S.D.

assumption. The brain in hepatic coma does not always remove ammonium from the blood but may in fact occasionally add to it, the jugular vein concentration exceeding that in an artery [*22*]. The cerebral uptake of ammonium is not greater in patients in hepatic coma than in cirrhotic patients without coma [*25*]. Cerebral oxygen consumption is reduced in patients with liver disease without evident neurological disturbance and does not correlate with the arterial ammonium level [*25*]. Finally, blood ammonium levels do not rise when neurological changes are precipitated by methionine [*11*].

Blood ammonium levels are frequently elevated in hepatic coma [*4,16,29–32*] but 10 per cent of values are in the normal range regardless of the degree of neurological involvement [*32*]. (Fig. 2.) The blood ammonium level shows some degree of correlation with the electroencephalographic changes [*33*]. Some patients in deep

coma may have normal values, and patients, particularly after portacaval anastomosis, may seem normal with raised levels. The amine oxidase inhibitor, marsilid® [*34*], or sodium glutamate [*35*] may reduce high blood ammonium levels in patients in hepatic precoma but clinical improvement does not always result.

At the present time it is impossible to decide whether the elevated blood ammonium levels reflect mainly the amount of portal venous blood reaching the systemic circulation without being adequately metabolised by the liver or whether ammonium is an important cerebral intoxicant in patients with liver disease. Other toxic substances of intestinal origin that are carried in the portal vein must be considered. These include the many pharmacologically active amines which can be produced by intestinal bacteria acting on protein [*20*]. Technical difficulties in the quantitative estimation of the very small amounts of amines present in blood have prevented investigation of this aspect as fully as it deserves.

CHANGES IN CARBOHYDRATE METABOLISM

In contrast to the hepatectomised dog, hypoglycaemic episodes are rare in chronic liver disease [*36*] but occasionally complicate fulminant hepatitis [*37*]. The blood glucose concentration in hepatic coma is usually normal.

The liver is intimately concerned with intermediate carbohydrate metabolism, and the raised blood keto acid levels reported in severe liver disease [*26*] might indicate failure of this process. Alpha ketoglutaric and pyruvic acids are transported from the periphery to the metabolic pool in the liver. Raised levels of alpha ketoglutaric acid in the blood have been reported in liver disease [*28*] and increases in both levels noted when there is complicating hepatic coma [*22,27*]. Mean levels increase as the neurological state deteriorates [*27*]. Values particularly for alpha ketoglutaric acid correlate better with liver cell function than with the extent of the collateral circulation. These results suggest progressive impairment of intermediate carbohydrate metabolism if the liver fails.

ELECTROLYTE CHANGES

The carbon dioxide combining power of the plasma is but slightly reduced and this is not constant. Roberts and her co-workers [*38*] have described a respiratory alkalosis evidenced by a high blood pH and a low PCO_2 level in patients with hepatic coma. Because of the characteristics of the oxyhaemoglobin dissociation curve in the presence of respiratory alkalosis, oxygen unloading would be impaired at the tissue level and this could diminish cerebral oxygen consumption. Respiratory alkalosis can result in neurological and electroencephalographic changes similar to those of hepatic precoma. This respiratory alkalosis might be due to retention of ammonium which stimulates the respiratory centre. Experimentally, alkalosis may also allow ammonium to enter the cell more readily [*39*].

Low serum potassium values are sometimes noted in patients with hepatic precoma [*40*] and improvement has followed potassium therapy [*41*]. In certain patients with cirrhosis, hepatic coma seems to be precipitated by sudden potassium depletion. This may be due to diarrhoea or ACTH administration. It may be one factor in the precipitation of coma by abdominal paracentesis. Diuretics, especially chlorothiazide, given to patients on a rigidly restricted sodium intake may result in hepatic precoma [*42*]. In patients with cirrhosis, hepatic precoma has followed a dietary regimen low in potassium with the addition of exchange resins [*43*].

Magnesium deficiency may be contributory, especially in alcoholic subjects and those maintained for long periods on parenteral glucose [*44*].

OTHER FACTORS

Urinary coproporphyrin excretion varies with the degree of hepatocellular failure, diminishing with coma and increasing with recovery. Retained porphyrins may play a part in the cerebral dysfunction [*45*].

Unconjugated bilirubin is lipophilic and high blood levels are believed responsible for the features of kernicterus complicating haemolytic disease of the newborn [*46*]. In the adult, unconjugated bilirubin rarely achieves such high values although, very occasionally, this may occur and these must be considered a rare contributory factor to cerebral dysfunction complicating deep jaundice.

Survival of the perfused brain depends on substances released by the liver into the circulation [*47,48*]. Infusion of the nucleosides, cytidine and uridine, corrected the faulty metabolism of the brain, suggesting that these were metabolites essential for the brain and synthesized by the liver. Hepatic failure may lead to serious deficiency of these and other such substances. Bessman and co-workers suggest that the pre-

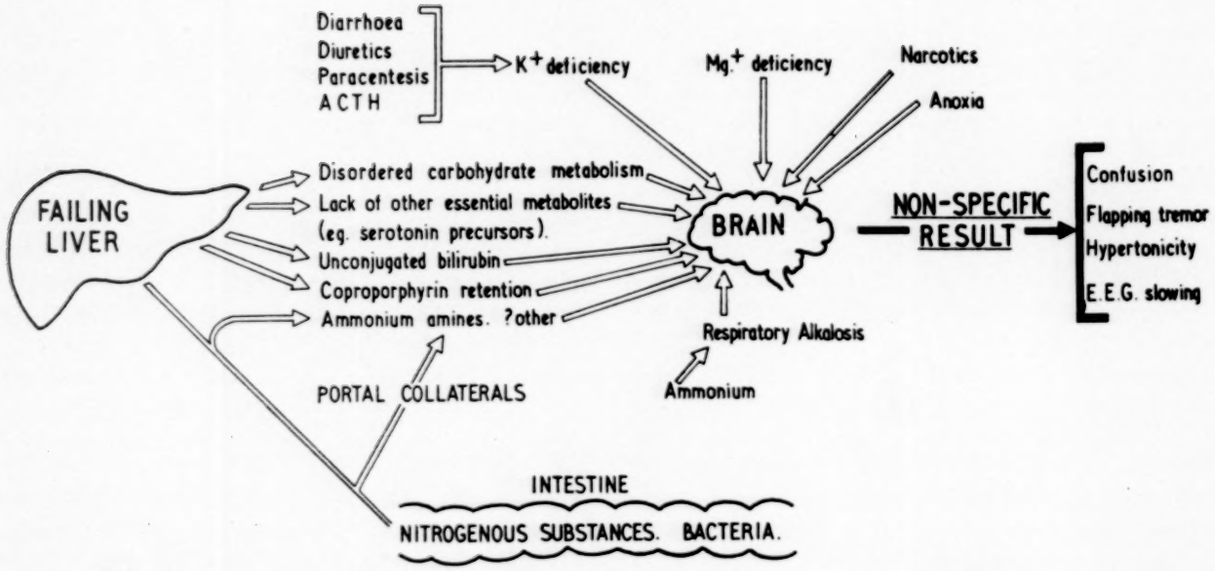


FIG. 3. A theoretical diagram illustrating the many factors that might alter cerebral metabolism in a patient with hepatic disease.

cursor for serotonin may be deficient [*49*]. Serial electroencephalographic records were taken in patients with hepatic precoma before and after parenteral injections of the precursor substance, 5-hydroxytryptophane (5 HTP), resulting in an increase in faster frequencies and a decrease in slow activity. These observations need confirmation.

STRUCTURAL CHANGES IN THE BRAIN

Grossly, the brain reveals little of importance. Microscopically, the characteristic change is diffuse enlargement to two to three times normal and proliferation of the protoplasmic astrocytes [*50*]. This change is diffusely found in the grey matter of cerebrum and cerebellum and in the putamen and globus pallidus. There is no conversion of protoplasmic to fibrous astrocytes. The nerve cells show relatively minor alterations. This combination, and the extent, seem virtually specific for liver disease; they are not observed in many other conditions, including cerebral anoxia, most instances of uraemia, in vitamin B deficiency, head injury or hypoglycaemia. The changes in the brain bear a rough relationship to the duration and severity of the hepatic coma and precoma, and can be expected to develop within a few days.

The significance of these changes in the pathogenesis of hepatic coma is difficult to evaluate. It is possible that they contribute to the easy response of the brain to toxic influences in persons with liver disease, particularly in those with chronic neuropsychiatric changes. This would certainly apply to patients with coincident cerebral disease such as arteriosclerosis. Permanent structural changes in the brain might be related to the failure of the chronic group, even when treated, to regain completely their previous mental status.

CONCLUSIONS (FIG. 3)

The picture of hepatic precoma and coma is in most respects non-specific. The organic dementia, the flapping tremor, the electroencephalographic changes and the raised blood ammonium values may be encountered in whole or in part in other disturbances such as uraemia, congestive heart failure or asphyxia. There is no sure laboratory method of diagnosis, and recognition depends on clinical acumen and the association with some of the other features of liver disease such as fetor hepaticus, jaundice or ascites. It is not justifiable to regard the syndrome as an entity of single causation. The reaction of the patient with liver disease to seemingly quite small metabolic changes in the brain, induced for instance by a small dose of morphia or by potassium depletion, is quite beyond that seen in persons with normal liver function. It is tempting to speculate that the brain of a patient with liver disease is peculiarly sensitive to metabolic insults. This might be related to the multiple, additive effect of the numerous toxic factors

affecting the brain in such a patient or to failure of the liver to provide some metabolite essential, perhaps in trace amounts, for cerebral metabolism.

TREATMENT

If it is accepted that hepatic coma is usually of multiple causation, then the first duty is to define the factors acting in the particular patient and to deal with them accordingly. The major known factor is the effect of toxic nitrogenous substances formed in the intestine by bacterial action on protein and this will be considered first.

Diet: All dietary protein is stopped. At least 1,600 calories are supplied daily as glucose drinks or as 20 per cent glucose through an intragastric drip. Twenty per cent or 40 per cent dextrose is given intravenously through plastic tubing introduced via an antecubital vein into the innominate vein or superior vena cava. Small veins will be thrombosed by glucose of this strength.

During recovery protein is added in 20 gm. increments on alternate days. The protein is divided between four meals. Any relapse is treated by a return to the former regimen. In patients with an acute episode of coma a normal protein intake is soon achieved. In the chronic group, permanent protein restriction is needed to control mental symptoms [*51*]; the limits of tolerance are usually 40 to 50 gm. per day. In this group an exacerbation of symptoms is treated by rest and abstention from protein.

In the acute cases, a few days to a few weeks deprivation of protein does not prove harmful; and even in the chronic group in which dietary protein has to be restricted for many months, clinical protein malnutrition is not seen [*35*]. These findings support the observation that in patients with liver disease nitrogenous equilibrium can be maintained on a severely reduced intake of protein [*52*], and the risk of temporary nitrogen depletion is preferable to the hazards of nitrogen toxicity. This measure, however, is indicated only in the patients showing signs of coma. Other patients with liver disease may benefit by high protein feeding, particularly if ascites is forming. Glucose provides a ready source of energy, and Jones [*53*] noted a 30 per cent fall in the mortality of acute hepatic insufficiency when intensive glucose therapy was used.

Vitamin K and B complex supplements are given parenterally.

Antibiotics: It seems reasonable to diminish bacterial action in the intestine by administering a wide-spectrum antibiotic. The tetracyclines have the disadvantage of causing diarrhoea and, with the emergence of resistant organisms, staphylococcal enterocolitis. They are certainly not suitable for long-term use. Neomycin given orally is very effective in decreasing gastrointestinal ammonium formation [*12*], an index of bacterial action on protein, resistant E. coli and staphylococci are uncommon, and little is absorbed. This is now the most satisfactory antibiotic for routine use in hepatic coma [*13*]. In the acute case, 6 gm. are given daily in divided doses. Results can be assessed more easily in the chronic portal systemic encephalopathies when the administration of neomycin is continued indefinitely in a dose of 4 gm. daily. It is useful not only in controlling symptoms when protein restriction fails, but also in allowing a higher protein intake [*12*]. The electroencephalogram improves, blood ammonium values fall and fetor hepaticus is abolished [*13*]. The stools show a changed flora, E. coli decreasing, resistant Strep. faecalis eventually appearing, and bacteroides being unaffected [*13*]. Clinical improvement is difficult to correlate with the faecal flora changes [*13*]. Patients have been continued on this treatment for as long as ten months with good results. Patients with progressive deterioration of liver function, however, may eventually prove refractory to treatment.

Neomycin is costly. Its routine use is essential in acute coma, but its continuation over many months may prove financially impossible. However, such patients with the chronic syndrome who find dietary protein restriction ineffective are rare, and it is difficult to withhold neomycin if it means the difference between chronic invalidism and productive work. Attempts must be made to withdraw the antibiotic although in many instances it will have to be resumed.

Purgation. Relapses with increasing coma may follow constipation, and remissions are associated with free bowel action. In the chronic group, drastic purgation can result in some improvement clinically and in the electroencephalogram, and also in a fall in the blood ammonium level [*13*]. The effect is not so dramatic, however, as with neomycin and cannot be sustained for the treatment is too vigorous for con-

tinued use. The value of enemas and routine purgation with magnesium sulphate in patients with hepatic coma must, however, be re-emphasized.

Glutamic acid therapy is based on the observation that glutamic acid reacts with ammonium, forming innocuous glutamine [*54*]. Good results have been claimed in small series [*54,55*] although this has not been confirmed by wider application under controlled conditions [*35,56,57*]. Certainly clinical results may be poor although blood ammonium levels may fall [*35*]. McDermott and co-workers [*58*] have reported good results in the group with coma due to gastrointestinal haemorrhage or intolerance of nitrogenous substances [*58*]. The results, however, were no better than in a comparable series treated only with protein restriction and chlortetracycline [*35*]. Failures were also reported by Webster and Davidson [*59*] although these authors found that large amounts of the substance (over 100 gm. daily) seemed to be of temporary benefit in some patients with cirrhosis. The results are not sufficiently convincing to recommend the routine use of sodium glutamate in hepatic coma. The inevitable infusion of large amounts of sodium with the glutamate may favour the development of pulmonary oedema.

Arginine is said to reduce the blood ammonium levels in hepatic coma [*60*]. Blood urea nitrogen values rose and the effect was presumably due to stimulation of the Krebs cycle in the liver. In many of the patients treated successfully the coma was precipitated by gastrointestinal haemorrhage or ammonium chloride, a group with a high spontaneous recovery rate. The administration of ornithine, another member of the Krebs cycle, was of little benefit in hepatic coma [*27*]. Arginine cannot be recommended for routine use.

Amino acids such as methionine are toxic to patients in hepatic coma. They cannot be metabolised by the liver and are already being excreted in the urine. Similarly lipotropes such as choline prove of no value and may be toxic.

Thioctic acid (lipoic acid) is a co-enzyme essential for the formation of active acetate and obligatory for the efficient working of the tricarboxylic acid cycle and hence for the metabolism of pyruvic acid. In view of the increased serum pyruvic acid values noted in hepatic coma, thioctic acid has been recommended for its treatment [*61*]. Results in four patients, however, were very disappointing [*27*].

PRECIPITATING FACTORS

Patients in impending coma are extremely sensitive to sedatives and, whenever possible, these are avoided. If an overdose is suspected, the appropriate antagonist such as N-allyl normorphine or megimide should be given. If the patient is uncontrollable, half the usual dose of barbitone is given; morphine is absolutely contraindicated. Drugs known to induce hepatic coma such as ammonium salts, ammonium exchange resins, methionine, urea, acetazolamide [*62,63*] and chlorothiazide are disallowed.

A common precipitant is gastrointestinal haemorrhage, and the gastric contents should be examined and a rectal examination performed in all patients when the precipitant of coma is uncertain. Gastrointestinal haemorrhage with hepatic coma is always an indication for the Sengstaken oesophageal compression tube. Blood transfusion is used to maintain the haemoglobin level above 10 gm. per 100 ml. Toxic products, including significant and increasing amounts of ammonium, develop rapidly in blood after shedding [*18*] and, whenever possible, fresh blood is used for transfusion.

Potassium deficiency may be present or may develop if electrolyte-free intravenous feeding is associated with a good urinary output. A high carbohydrate intake is particularly liable to depress the serum potassium value. With oral feeding, this can be treated by liberal administration of fruit juices or, if the serum potassium level is low, by means of 2 gm. potassium chloride administered orally three times daily. Intravenous administration of potassium should be avoided but if it is imperative 0.1 per cent potassium chloride may be incorporated with the 20 per cent glucose and infused at a slow and unvarying rate to avoid myocardial toxicity.

The possibility of the development of delirium tremens may tempt the clinician to give alcohol to a patient in impending hepatic coma who has recently been drinking heavily. This is rarely necessary and alcohol should be completely withdrawn. The development of delirium tremens is an indication for small doses of serpasil® or chlorpromazine.

THE PLACE OF CORTICOID HORMONES

This therapy has met with little success in the hepatic coma complicating cirrhosis [*64,65*] and is not indicated. In virus hepatitis the outlook for the comatose patient is so poor that a case can

be made for steroid therapy. Favourable results have been reported [*66*] and also failures [*67*]. In fulminant hepatitis it is probably wise to use hydrocortisone intravenously in large doses (100 mg. six hourly). The steroid does not seem to affect the underlying disease process but serves only to "buy time" so that the enormous regenerative capacity of the liver will be able to exceed necrosis. I have given this therapy to six patients with hepatic coma due to virus hepatitis; four died and two recovered, other lines of treatment were being used simultaneously.

PRACTICAL MANAGEMENT OF COMA [*68*]

1. Grade neuropsychiatric status four-hourly. *Grade 1:* minor disorders of consciousness and the motor system. *Grade 2:* gross disorder of consciousness with disorientation in time and space. *Grade 3:* coma.
2. Keep daily handwriting chart. Daily tests of simple arithmetic and power to copy a five-pointed star in matches [*69*].
3. Chart temperature and pulse four-hourly.
4. Chart depth of jaundice by serum bilirubin levels every three days.
5. Assess hepatic size daily.
6. Examine lungs carefully daily for evidence of infection or oedema.
7. Chart fluid intake and output. Weigh daily if coma is not deep.
8. Determine haemoglobin level and packed cell volume and occult blood examination of faeces daily, as a guide to gastrointestinal bleeding and an indication for blood transfusion.
9. Determine serum sodium, potassium and bicarbonate levels daily to assess electrolyte equilibrium.
10. Determine blood urea level daily especially if there is oliguria.

PROGNOSIS AND RESULTS OF TREATMENT (TABLE I)

Prognosis depends on the extent of liver cell failure. The chronic group with relatively good liver function but with an extensive collateral circulation combined with increased intestinal nitrogen have the best prognosis, and the acute hepatitis group the worst. In cirrhosis the outlook is poor when the patient has ascites, jaundice and a low serum albumin level, all indicative of poor liver cell function [*35*]. If treatment is begun early in the precomatose state, the chances of success are increased.

Hepatic coma has been divided into endogenous (patients with advanced liverdis ease without a clear precipitant) and exogenous (patients with reasonably well compensated liver disease in whom precipitating factors can be incriminated) [*58*]. This is not a clear-cut classification, for neuropsychiatric features and electroencephalographic records are identical and there is no diagnostic biochemical differentiation between the groups. A multiplicity of precipitating factors may occur in the same patient, and gastrointestinal haemorrhage impairs liver function as well as increasing nitrogen in the intestine. Haemorrhage is also common in patients already comatose. It is not surprising, however, that the prognosis is better in the group with a clear precipitant; for this indicates a positive line of therapy.

TABLE I

HEPATIC COMA (MODIFIED WITH ADDITIONS FROM THE DATA OF SHERLOCK, SUMMERSKILL AND DAWSON [*35*])

Disease	Total	No. Reaching Grade 3	Deaths
Acute virus hepatitis	19	12	9
Cirrhosis*			
Acute	56	44	30
+ Gastrointestinal haemorrhage†	16	14	10
Nil	14	11	9
Paracentesis abdominis	9	6	4
Surgical operation	7	6	3
Acute alcoholism	3	2	0
Portal vein thrombosis	3	3	3
Ammonium chloride	2	0	0
Infection	2	2	1
Chronic	27	17	0
Extrahepatic portal vein block + precipitant	3	0	0
Chiari's syndrome	2	2	2
Low output cardiac failure; hepatic necrosis	1	1	1
	108	76	42

* Only five patients suffered from biliary cirrhosis.

† The major precipitant is given. Multiple factors were usually present and these often included morphia and barbiturate administration. Patients in the chronic cirrhotic group had a large portal collateral circulation and in all instances hepatic precoma could be precipitated by a high protein diet.

Assessment of therapeutic results in hepatic coma is complicated by the unpredictable fluctuations in its clinical course. Investigations should therefore be conducted on large series of patients on controlled regimens, with recovery (defined as ability to be discharged from hospital) as the sole criterion of success. Patients with chronic portal systemic encephalopathy (chronic intermittent stupor) must be separated from the acute group; for in these patients, provided oral protein is withheld, spontaneous recovery is usual.

The therapy of hepatic coma is only one facet of the whole management of the patient with chronic liver disease. The chances of another complication developing or of coma recurring are considerable. The majority of survivors, however, are alive one year later and treatment is eminently worthwhile. This is particularly true of the group with acute hepatitis in which recovery, if it occurs, is usually complete and not followed by cirrhosis. The regimen described pays particular attention to the control of toxic nitrogenous substances passing from the intestine to the brain; this is only one aspect of liver failure. Electrolyte and other metabolic changes must be occurring when the liver fails. This conservative regimen is suggested as the best available and will be modified when other effective remedies are discovered.

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Case Reports

Coronary Heart Disease in Identical Female Twins*

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ALTHOUGH coronary heart disease is a common condition, to the best of my knowledge no more than six instances of this disorder in identical male twins have been published in the American and Western European literature [*1–4*]. Only four of these can be accepted as proved instances of coronary disease in both twins, since in cases E.Z.5 and E.Z.13 of Kahler and Weber there is convincing evidence of the presence of the disease in only one of each pair of twins [*3*]. I have found no previous description of the disease in female twins. It therefore seemed worthwhile to report the following instance of coronary insufficiency in uniovular twin sisters in their early forties. Their case is of further interest because of the low incidence of the disease in women of this age group who do not have diabetes, hypertension or familial hypercholesterolemia.

CASE REPORT

The twins, C. D. and A. R., were born on a farm in the Mid-west in February, 1914. They have always looked so much alike that even today it is almost impossible to tell them apart. The close similarity in their general appearance, features, hair, eye and skin color and texture, and in other aspects conforms to the criteria of Newman for the recognition of identical twins [*5*]. They have one older and four younger healthy brothers. Their parents are unrelated. The father died recently at seventy-five years of age of an "enlarged heart" without evidence of clinical coronary thrombosis. The mother is seventy years old and well. A maternal uncle had a "heart attack" at forty years of age and died of an enlarged heart at seventy-five. A second cousin died of long-standing diabetes and kidney trouble at thirty-five years. Otherwise there is no family history of heart disease, hypertension or diabetes.

The twins have always been close to each other. After their childhood on the farm they attended college together and majored in related fields. C. D. was considered a little stronger and more self-reliant than her sister. She has remained single, and has worked as a restaurant manager in Washington since 1942. I first saw her in 1944 for a kidney stone which she passed successfully. Since then she has had no further renal trouble. In 1953 she had a hemithyroidectomy for nodular goiter, and a biopsy of the breast which showed fibrocystic mastopathy with sclerosing adenosis. In January, 1955 she was seen for a routine check-up. On persistent questioning, it was learned that during the past year she had noted occasional, non-radiating, high substernal tightness with exertion, hurry and excitement. The sensation, which was more apt to occur in the cold, disappeared promptly when she stopped the activity which brought it on. She had also noted a little shortness of breath with exertion. The remainder of the history was negative except for the complaint of premenstrual tension.

She was a well developed forty year old white woman who was 5 feet 3¼ inches tall and weighed 124 pounds. A pea-sized nodule was noted in the upper pole of the right thyroid lobe, and the breasts were diffusely nodular. Examination of the heart showed the point of maximum impulse to be in the fifth intercostal space within the midclavicular line. The heart sounds were good. The aortic second sound was slightly greater than the pulmonic second sound, but it was not accentuated. The pulse was 80 and regular, and the blood pressure was 105/70 mm. Hg. The rest of the examination was within normal limits. Blood count and urinalysis were normal. A chest x-ray at a somewhat later date showed that the heart was normal in size. The ascending aorta was somewhat elongated and tortuous. A resting electrocardiogram at the end of February showed low, slightly diphasic T waves in V_2 and V_3 and was otherwise within normal limits. (Fig. 1.) The single Master two-step test was positive with a flat T_1 and an inverted T_{V4} after exercise. When she was seen again in early March she stated that she had had daily chest pain on exertion and

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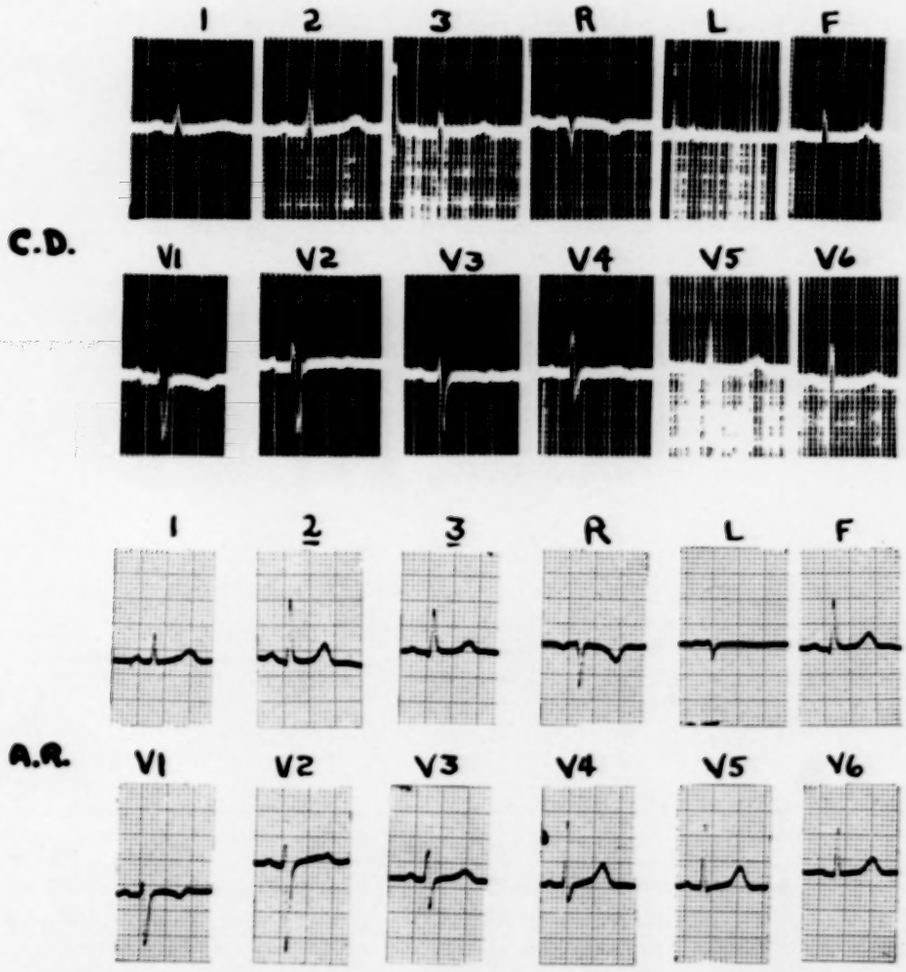


FIG. 1. Electrocardiograms of C. D. and A. R. before onset of coronary insufficiency.

hurry. The pain lasted fifteen minutes after cessation of activity and was more frequent during the premenstrual period. At this time her resting electrocardiogram showed flat T waves in leads I and V_5 and inverted T waves in V_1 through V_4. (Fig. 2.) Since she had no one to care for her at home, she was hospitalized for three weeks with a diagnosis of coronary insufficiency. Although she felt rather discouraged and apprehensive while in the hospital, her course there was uneventful. After discharge she regained her strength and energy slowly. At present she is working full time at her rather taxing job. She still has occasional angina on exertion, but is again engaging in a fair amount of outside activity. Her cardiogram has reverted to its original configuration. (Fig. 3.)

A. R. and her husband came to Washington in 1942. At this time she was found to have a large uterine fibroid, and a subtotal hysterectomy was performed. I first saw her in 1943, prior to the adoption of a baby boy. Some years later her husband became seriously ill with a chronic relapsing disease of which he eventually died in 1954. During this difficult and anxious period she returned to work in order to secure her future. Her job was arduous, poorly paid and unpleasant. In 1953 a left hemithyroidectomy and biopsy of the breast was performed. The pathological findings were similar to those noted in her sister. In 1955, about six months after her husband's death, she was further upset by her sister's illness and feared that the same fate might lie in store for her. At this time her history was negative. On physical examination she was 5 feet 2½ inches tall and weighed 122 pounds. Her breasts were diffusely nodular. The point of maximum impulse of the heart was in the fifth intercostal space within the midclavicular line. The heart sounds were good. A_2 was somewhat greater than P_2. The pulse was 80 and regular, and the blood pressure was 105/80 mm. Hg. Chest x-ray showed a normal heart with a somewhat dilated ascending aorta. The resting electrocardiogram was normal. T_{V2} and T_{V3} were upright. (Fig. 1.) The single Master two-step

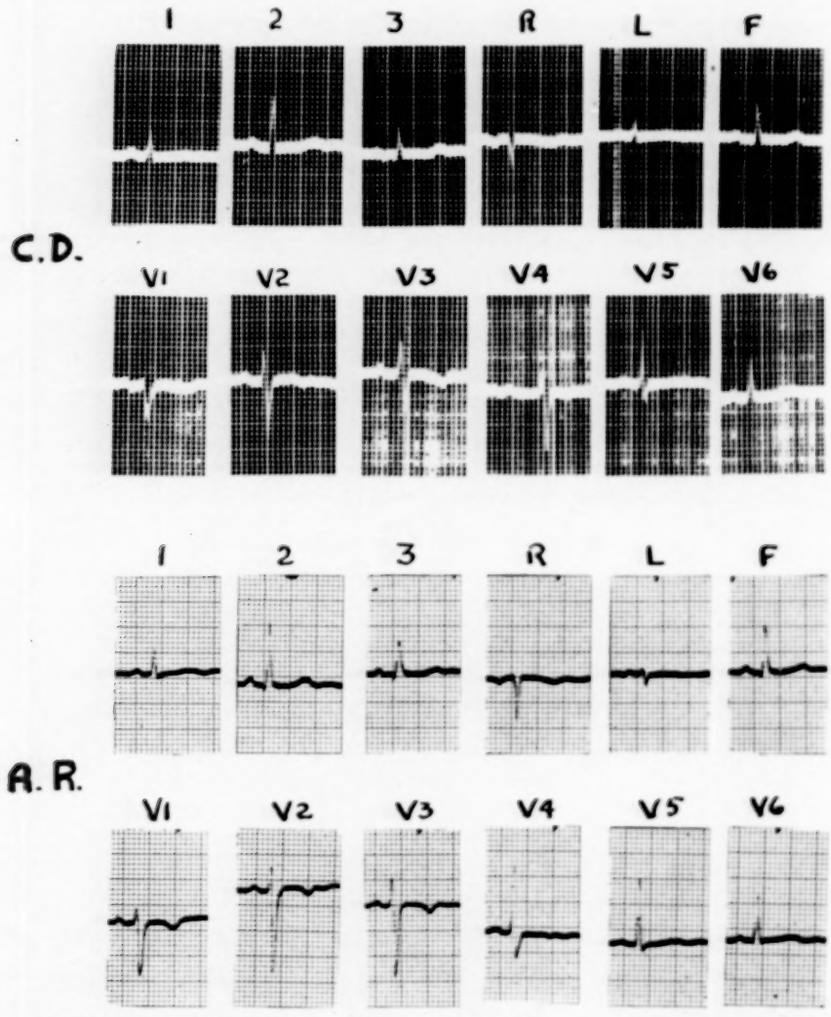


FIG. 2. Representative electrocardiograms of C. D. and A. R. during period of coronary insufficiency.

test was normal, but the double test showed ST depressions of 0.5 mm. in lead I and of 0.9 mm. in lead II. Blood count and urine examinations were normal.

Toward the end of August, 1955, she first noted substernal "tiredness" which occurred after, but not during, exertion. This lasted about half an hour at a time. On August 30 it had been present all morning. There was no change in the physical examination. The electrocardiogran was within normal limits, but T waves in the limb leads and over the precordium were lower than they had been. When she was seen again on September 9 she had been feeling weak, tired and anxious. No classical anginal pain had been present, but she had noted an "uncomfortable uneasy" feeling in the substernal area when tired. She also complained of air hunger with sighing respiration, intermittent palpitations and cold sweaty hands. The electrocardiogram now revealed generally low T waves. TV_1 through V_3 were inverted and TV_4 was flat. (Fig. 2.) She was put to bed for three weeks, and then slowly resumed her activities. During this time her cardiograms showed accentuation and then a partial regression of the T wave changes. After seven weeks at home she started on a better paid and more satisfactory job. Her physical progress was complicated by the accentuation of previously present anxiety symptoms. These were ameliorated by three months of supportive psychotherapy. At present she is getting along well but still tires easily. All findings are normal except for slightly diphasic T waves in V_2 and V_3. (Fig. 3.)

FURTHER TESTS

Displacement, velocity and acceleration ballistocardiograms with the modified Dock technic using the Smith-Perls instrument were performed on both twins by Dr. John E. Smith and were found to be normal. Protein-bound iodine value was 5 mg. per cent in C. D. and 5.1 mg.

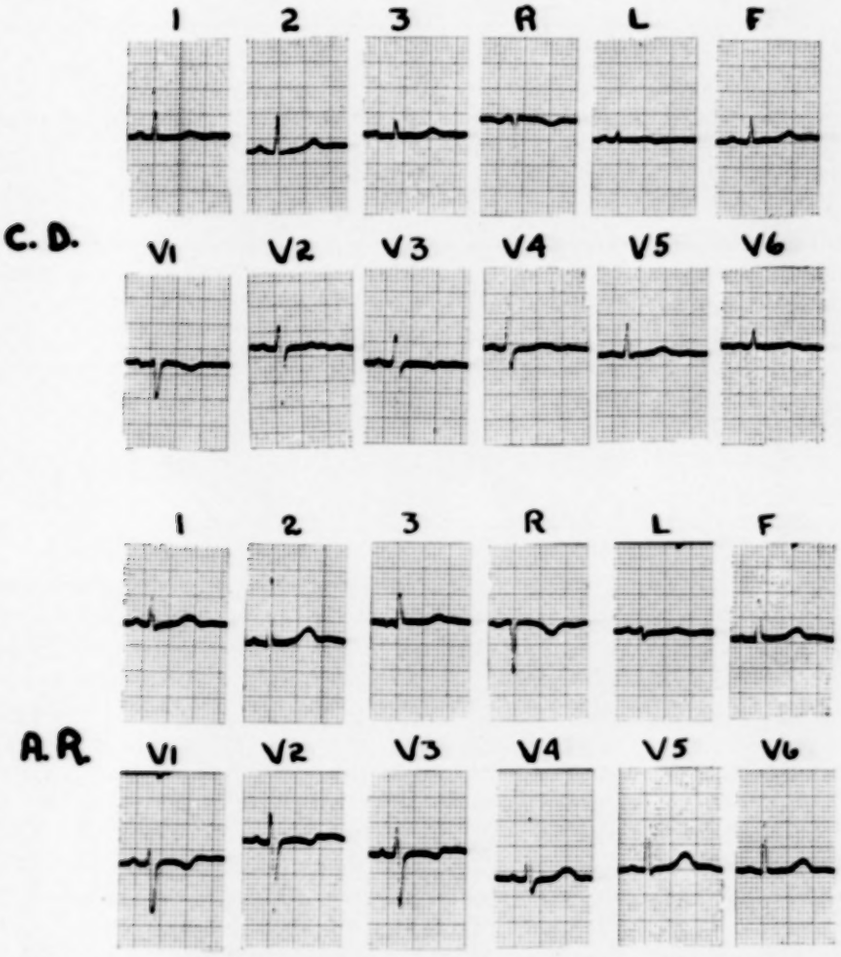


FIG. 3. Representative electrocardiograms of C. D. and A. R. one year after recovery from coronary insufficiency.

per cent in A. R. A glucose tolerance test was normal in C. D. and somewhat hypoglycemic in A. R. Cholesterol and lipid determinations, performed by Dr. Harold Orvis, were in the high normal range for his laboratory.

Tests	Patient	
	C. D.	A. R.
Serum total lipid (Sperry and Brand)	612 mg.%	787 mg.%
Cholesterol (Sperry and Webb)	211 mg.%	230 mg.%
Cholesterol esters	148 mg.%	158 mg.%
Phospholipids (Fiske and SubbaRow)	250 mg.%	300 mg.%
Cholesterol/phospholipid ratio	0.84	0.77
Per cent beta lipoprotein (Langam, Durrum and Jenks)	70.6%	75%

COMMENTS

The history, physical findings, laboratory studies and clinical course of the disease in these twins show many similarities. Unlike the previously reported four cases of coronary disease in identical twins, in which all patients exhibited mild to moderate hypertension and one each of two sets were known to have diabetes, neither of these women had high blood pressure or diabetes. C. D. noted anginal pains for more than a year before the onset of coronary insufficiency, and has continued to experience mild angina since then. A. R. complained of vague chest discomfort but has never noted typical angina pectoris. Despite this absence of symptomatology, she had coronary insufficiency six months after her sister. In both women the

episodes were clinically mild, and in both they were followed by many months of weakness and easy fatigability. It is especially interesting that A. R. had lived through years of anxiety in connection with her husband's illness prior to the onset of her own disease. During this time she had also felt harassed by a harsh driving supervisor, and she had had many problems connected with the rearing of her boy. She had always been the more dependent of the twins, and overt anxiety symptoms had developed after her husband's death. Despite these multiple strains she became ill some time after her sister, and her sickness has not been more severe.

In two of the previously reported cases of coronary heart disease in uniovular twins the course of the illness was also very similar in each pair [*1,4*]. In the other two the information given is less complete, and the similarity in each pair seems less striking [*3*].

Physical examination of the sisters showed a close correspondence in their height, weight, blood pressure and cardiac findings. On chest x-ray there was some tortuosity of the ascending aorta in each twin. The heart configurations were similar, but C. D.'s heart was somewhat fuller than A. R.'s. Comeau and White [*6*] have noted a close correspondence in heart size and configuration in identical twins.

The twins' original electrocardiograms resembled each other but were not identical. A. R.'s tracings have consistently showed higher voltage, especially in the limb and unipolar leads. C. D.'s first tracing, which was taken at a time when she may already have been suffering from coronary insufficiency, showed low, slightly diphasic T waves in V_2 and V_3, while these waves were upright in A. R.'s first record. Master tests were positive in both, but a double test was needed to bring out ST depressions in A. R., while a single test produced T wave inversion in C. D. Electrocardiographic changes during the episodes of coronary insufficiency showed analogous T wave abnormalities in both twins. Each now has a normal electrocardiogram except for the previously described T wave changes in V_2 and V_3.

As far as can be ascertained from the descriptions of electrocardiograms in the previously reported cases, the similarity of the tracings in each set of twins was not especially striking. However, the electrocardiographic studies in these cases were rather meager. Wise and colleagues [*7*] studied electrocardiograms in identical and fraternal twins with normal hearts. Almost 50 per cent of the uniovular twins had closely similar tracings, while another twenty-five per cent showed some similarity in their records. In biovular twins, on the other hand, tracings were closely similar in less than 25 per cent and showed no resemblance in 50 per cent.

It is of interest that each twin had a normal ballistocardiogram. In Dr. Smith's experience only 10 per cent of patients with proved coronary artery disease are free of abnormalities by this examination.

Cholesterol and lipid studies were in the high normal range in each sister. A. R.'s values were consistently higher. Cholesterol values previously reported in sixteen year old [*8*] and ninety-nine year old [*9*] uniovular female twins were practically identical. In the ninety-nine year old women, phospholipids and serum protein fractionations were also closely similar. On the other hand, in a pair of fifty-five year old identical male twins exhibiting xanthoma tuberosum without clinical coronary disease the cholesterol value was 414 mg. per cent in one and 463 mg. per cent in the other [*10*].

Many investigators have suspected a hereditary element in coronary disease but its existence remains unproved. A systematic investigation of the illness in a series of identical and fraternal twins could shed considerable light on the subject. Kahler and Weber [*3*] attempted such a study for all forms of heart disease. Unfortunately, their patients with coronary disease were few in number and inadequately studied. The main value of their work lies in the number of cases which it contributes to the literature, but no definitive conclusions about heredity in the disease can be drawn from it. While individual case reports such as the present one can not prove the presence of heredity in coronary artery disease, they are sufficiently suggestive to raise the strong suspicion of its presence.

SUMMARY

This report deals with the first instance of coronary heart disease in identical female twins to be reported in the American and Western European literature. Coronary insufficiency developed in their early forties. Pertinent findings relating to their condition showed a striking similarity of the disease picture in each.

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Acute Disseminated Coccidioidomycosis*

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COCCIDIOIDOMYCOSIS is usually an acute pulmonary infection with a good prognosis. When the disease becomes disseminated, however, it is a chronic, progressive and often fatal condition. During the last eighteen months there have been nine cases of disseminated coccidioidomycosis treated at the William Beaumont Army Hospital. In all but one of these cases the course was prolonged, with death or recovery occurring after many months. The following is a case report of a patient who died of disseminated coccidioidomycosis two weeks following the onset of symptoms.

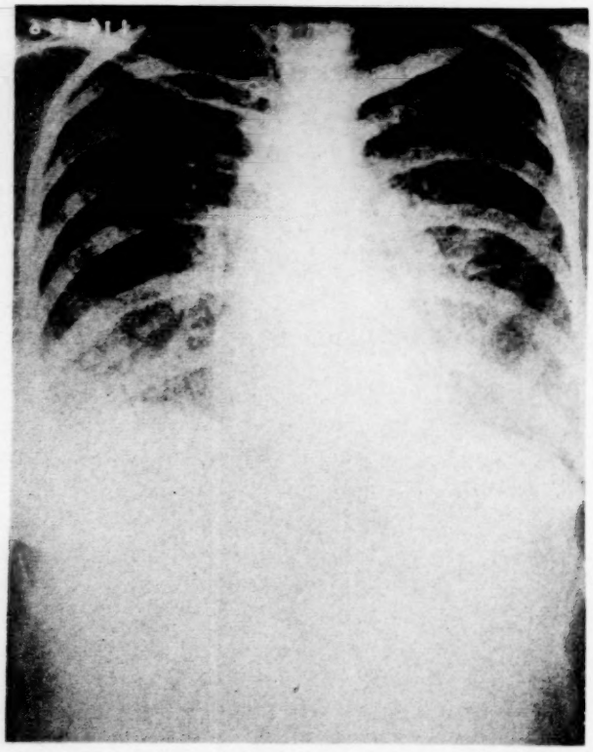


FIG. 1. Posteroanterior film of the chest taken on March 25, 1957.

CASE REPORT

B. T. M., a twenty-one year old white, married housewife was first seen at the William Beaumont Army Hospital on March 25, 1957. Ten days previously she had first noted a frequent, hacking, nonproductive cough which persisted until the time of admission and was associated with marked exertional dyspnea. The patient became short of breath on walking one block. She had had an occasional "chilly feeling" but no chills. Because of these symptoms the patient came to the general medicine clinic on March 25, 1957. A chest x-ray at that time revealed a fine granular infiltration throughout both lungs, and she was admitted for evaluation and therapy. There was no history of recent exposure to any contagious disease, toxins or to parakeets.

The past history was not remarkable. The patient had lived in Cleveland, Ohio, until August 1956, when her husband entered the U. S. Army and was stationed at Fort Bliss, Texas. The patient was six months pregnant. She had had no difficulties during the pregnancy and a routine prepartum chest x-ray on December 3, 1956 was normal. The family history was of interest in that a paternal grandfather had had pulmonary tuberculosis, but the patient had not been recently exposed. There was no other family history of tuberculosis or any other pulmonary disease.

Physical examination disclosed an acutely ill twenty-one year old white woman in marked respiratory distress. Her temperature was 104°F., pulse was 110 per minute and regular, respirations were 40 per minute and her blood pressure was 120/75 mm. Hg. Cyanosis was not present on admission. Positive physical findings included equal, bilateral respirations with some dullness at both bases posteriorly and fine rales at both bases posteriorly and laterally. Except for the tachycardia, the heart was normal. No pericardial friction rubs were heard. The uterus was enlarged and palpable at the umbilicus. The liver was tender and palpable one fingerbreadth below the right costal margin. The remainder of the physical examination was normal.

Laboratory examination revealed a white blood cell count of 9,600 per cu. mm. with 89 per cent neutrophils, 10 per cent lymphocytes and 1 per cent eosinophils. The hemoglobin was 11.4 gm. per 100 ml., and the hematocrit was 38 per cent. The urinalysis was normal. No pathogens were isolated on sputum or throat culture. The serological test for syphilis was negative. Two L.E. preparations were negative. PPD No. 1, PPD No. 2, coccidioidin 1:1000 and

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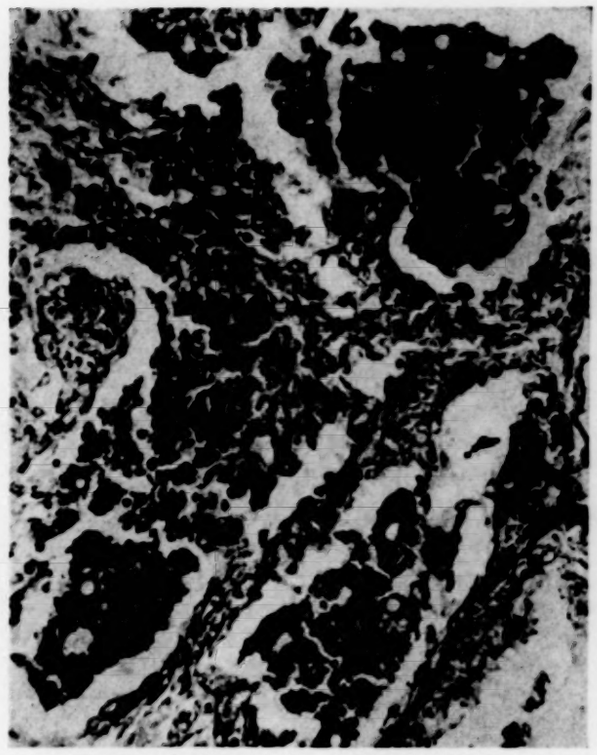


FIG. 2. Section of the lung obtained at autopsy enlarged 150 times. Note the spherical masses containing endospores which are the fungus C. immitis.

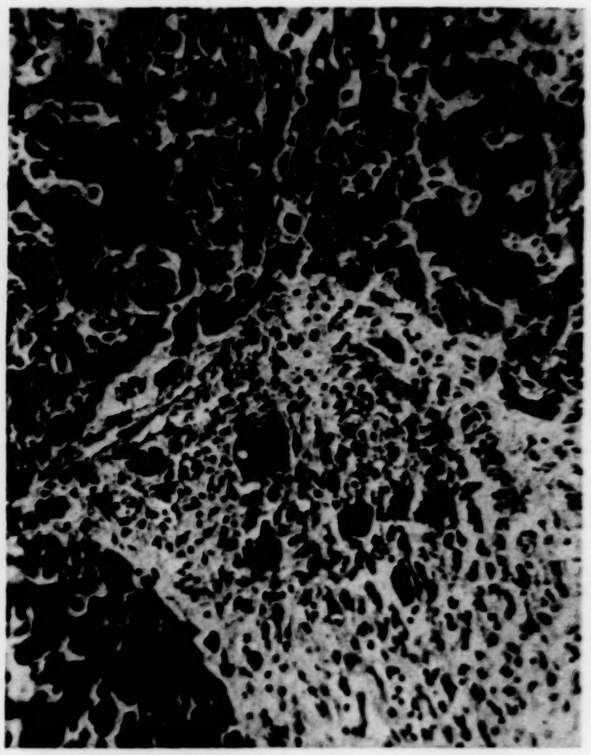


FIG. 3. Section of the liver obtained at autopsy enlarged 150 times. Note the spherical form of the fungus in the Langhans' giant cells.

1:100, and histoplasmin were negative. The blood carbon dioxide was 25 mEq./L., chloride was 100 mEq./L. The chest x-ray (Fig. 1), taken just before admission, was read as showing, "fine, granular appearing infiltrations, uniformly distributed throughout both lungs. There is no evidence of pleural effusion or hilar adenopathy. The heart and aorta appear normal." The impression of the radiologist was that the chest x-ray was suggestive of miliary tuberculosis but that histoplasmosis, coccidioidomycosis and sarcoidosis should be considered.

Three laboratory results, which were not available until after the death of the patient, were a urine and sputum culture, both of which grew out Coccidioides immitis, and a complement fixation test for coccidioidomycosis which revealed a titer of $\frac{1}{16}$. There was no titer found against either histoplasmosis or blastomycosis.

On admission the patient was placed on bedrest, isolation; 600,000 units of procaine penicillin was administered twice daily and 250 mg. of terramycin® four times daily. The next morning her fever persisted and she had minimal cyanosis of her lips and nailbeds. She was placed in an oxygen tent. The venous pressure was 140 mm. of saline solution and the circulation time was twelve seconds arm to tongue.

On the third hospital day a repeat chest x-ray was unchanged and the patient's temperature remained between 103° to 104°F. She received a trial of aminophylline, 0.5 gm. administered intravenously, without any change in her respiratory distress. She was started on a regimen of streptomycin 1 gm. per day and isonized 100 mg. three times a day, and was given 400 mg. of hydrocortisone intravenously. On the fourth hospital day the patient showed slight improvement, her temperature going down to normal and her dyspnea decreasing somewhat. She was given 80 mg. of metacortin® orally that day. On the fifth hospital day the patient was again febrile and cyanotic. By 3:00 P.M. she was cyanotic, even in the oxygen tent, and did not improve when nasal oxygen was started. Respiratory difficulty increased and she died at 6:30 P.M. on March 30, 1957.

At autopsy an intrauterine pregnancy of approximately twenty-four weeks was found. The placenta and fetus were normal. The ovaries were normal and on section a small corpus luteum cyst was seen. The lungs weighed 1,250 gm. each and there was some fibrinous exudate over the pleural surfaces. On section, there were many yellowish white nodules measuring approximately 1 mm. in diameter uniformly distributed throughout the lobes of both lungs. The gross appearance looked like miliary tuberculosis to the pathologist. On microscopic examination, however, the alveoli were filled with eosinophilic amorphous material together with polymorphonuclear leukocytes, macrophages and Langhans' giant cells. Within the giant cells were seen spherical masses containing

endospores consistent with C. immitis. (Fig. 2.) Although the liver did not appear abnormal on gross examination, on microscopic section granulomatous lesions were present containing spherical bodies consistent with C. immitis. (Fig. 3.) Microscopically the kidneys, adrenals and spleen were also involved by the fungus.

COMMENTS

In the white race, dissemination occurs in about one of five hundred cases of coccidioidomycosis, but it occurs fourteen times as frequently in Negroes and 180 times as frequently in Filipinos [*1*]. Pregnant patients seem to have a greater tendency to dissemination [*2*]. Although almost any organ of the body can be involved, in our patients, skin and bone involvement has been most frequent. In the disseminated form of the disease the skin test is usually negative; indeed, if it becomes positive, it is usually considered a good prognostic sign [*3*]. The diagnosis of disseminated coccidioidomycosis can be made antemortem by biopsy and culture of an extrapulmonary lesion, or by a complement fixation titer of $\frac{1}{32}$ or greater. The titer of $\frac{1}{16}$ found in this patient was suggestive but not diagnostic of dissemination [*4*]. Once dissemination has occurred the patient has, at best, a fifty-fifty chance of survival. Death usually occurs following a long, debilitating illness, during the course of which the patient becomes cachectic and multiple draining abscesses may develop. To date no effective therapy has been found, although a new drug, amphotericin B, is being tried here and in other hospitals in the endemic area to see if it is of value. Unfortunately, this patient was admitted before we had a supply of the drug.

SUMMARY

A report is presented of a young woman who died two weeks after the onset of symptoms of disseminated coccidioidomycosis.

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Diffuse Interstitial Fibrosis of the Lungs*

Successful Treatment of a Case, with Adrenal Steroids

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ONLY thirty-nine cases of diffuse interstitial fibrosis of the lungs (Hamman-Rich syndrome) have been reported since the original descriptions by Hamman and Rich in 1935 [*1*] and 1944 [*2*]. This is a disease of unknown etiology in which progressive and unremitting dyspnoea is a constant symptom; other features which are frequently present are cough, haemoptysis, cyanosis, clubbing of the fingers, loss of weight and right heart failure. The onset may be insidious or acute. With the exception of one instance [*3*] in all cases reported so far the patients have died from either respiratory or cardiac insufficiency. Those with an acute onset have usually died in a few weeks or months, while many of those with more chronic disease have survived for periods up to nine years. The histological features are a widespread connective tissue hyperplasia throughout the interstitial structures of the lungs, accompanied in places by metaplasia of the alveolar epithelium and the formation of alveolar hyaline membranes. The proliferative fibroblastic reaction in the alveolar walls seen in the early stages of the disease is replaced by collagen later on. Bacteria or other specific etiological agents are not in evidence and the alveoli themselves contain little if any cellular inflammatory exudate, although eosinophils may be seen in the interstitial tissues. These findings are by no means specific as identical changes may be found in the pneumoconioses, beryllium poisoning, organising pneumonia and in the so-called collagen diseases. The clinical and pathological aspects of diffuse interstitial fibrosis of the lungs have been fully discussed recently by Peabody et al. [*4*] and by Grant et al. [*5*].

This report concerns a case of diffuse interstitial fibrosis of the lungs which assumed a very acute form and in which a remission lasting three and one-half years has been induced by adrenal steroid therapy. Respiratory function studies were performed throughout the course of the illness. Isolated studies indicate that the characteristic physiological defect is a disturbance of diffusion, but assessments of respiratory mechanics have not previously been reported, nor are we aware of any long term physiological studies in cases under treatment.

CASE REPORT

On December 4, 1953, a twenty-one year old unmarried woman, consulted one of us (S. J. F.) complaining of cough and shortness of breath. She had been well until March of that year when a cough developed productive of a small amount of yellow sputum. She was treated with chlortetracycline without benefit. Two months later, because of persistence of the cough, her chest was x-rayed; no abnormality was found. The cough gradually abated but she then noticed breathlessness on exertion which became progressively worse during the ensuing months. Five months later the cough returned and she received several injections of penicillin followed for a week by sulphadiazine therapy without improvement in her symptoms. In November, 1953, she was again x-rayed because of pain in the right side of the chest and recurrence of the cough. The film showed appearances compatible with an incompletely resolved pneumonia at the right lower zone.

The physical examination was essentially negative with the exception of a temperature of 99.2°F., a pulse rate of 100 per minute and fine crepitations over the right lower lobe. The sedimentation rate was 15 mm./hour (Westergren). Examination of the sputum showed no tubercle bacilli. An x-ray film of the chest showed fine linear streaking at the right lower zone but no other definite abnormality. Bronchiectasis was suspected and she was admitted to hospital five weeks later for further study. Her condi-

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TABLE I

LUNG VOLUME MEASUREMENTS, MIXING AND EXERCISE TESTS

Date	Lung Volume and Subdivisions					Lung Mixing Index	Hugh-Jones Test				O_2 % Saturation‡		
	Vital Capacity		Residual Volume		Residual Volume/ Total Lung Capacity (× 100)		Resting Ventilation†	Exercise Ventilation†	Standard Ventilation†	Dyspnoeic Index	At Rest	Fall on Effort	Rise on O_2
	(L.)	% Predicted*	(L.)	% Predicted*									
March 15, 1954 (first relapse)	2.9	69	1.22	68	29	2.26	10.6	40.4	46.5	44	96	− 7.5	+ 9
April 25, 1955	2.7	64	.97	54	26	2.06	11.0	41.9	46.6	40	89	−41	+10
June 4, 1956 (treatment stopped)	3.62	86	1.53	79	28	2.23	12.0	54.0	61.0	43	96	− 5	+ 7
September 4, 1956	3.14	75	1.83	101	37	1.90	8.7	43.4	44.8	33	95	−10	+ 8
October 2, 1956	3.14	75	1.58	88	33	1.70	11.0	42.0	....	34	93	− 7	+ 8
December 5, 1956 (second relapse)	2.85	68	1.44	80	34	2.23	10.2	46.0	52.0	41	94	−11	+ 9
January 11, 1957	2.62	62	1.50	83	36	2.18	10.71	42.7	44.2	36	86	−19	+13
January 19, 1957	....	..	....	...	..	....	.....	....	....	..	93	− 8	+ 7
January 22, 1957	....	..	....	...	..	....	.....	....	....	..	93	− 8	+ 7
January 30, 1957	....	..	....	...	..	....	.....	....	....	..	92	− 9	+ 8
April 11, 1957	....	..	....	...	..	....	.....	....	....	..	93	− 6	+ 7

* Normal values taken from data of Goldman (1956).
† L./minute, B.P.T.S.
‡ Waters Conley Absolute Reading Oximeter.

tion had deteriorated markedly by that time in that she had become dyspnoeic at rest, the pulse rate had increased to 132 per minute, clubbing of the fingers and cyanosis of the tongue had become evident, and the crepitations had extended to involve most of the right lung and the lower lobe of the left. A bronchogram of the right lung only and bronchoscopy revealed no abnormality. On the next day cyanosis and dyspnoea had increased markedly and a gallop rhythm was heard at the xiphisternum. An electrocardiogram showed gross inversions of the T wave in leads 1, 2, AVF and in all the V leads with a deep S in leads 1 and AVL and a tall R in V1. (Fig. 1A.) The pattern was regarded as indicating severe cor pulmonale. No significant change had occurred in the x-ray appearances of the chest. A blood count showed a marked neutrophil-leukocytosis (hemoglobin 15.9 gm. per cent, leukocytes, 30,000 per cu. mm.; neutrophils, 82 per cent; monocytes, 3 per cent; lymphocytes, 14 per cent, basophils, 1 per cent). A provisional diagnosis of Hamman-Rich syndrome with acute cor pulmonale was made and treatment with erythromycin and cortisone was started. The dose of cortisone was 300 mg. on the first day, 200 mg. on the second day and 125 mg. daily thereafter. There was considerable general improvement during the following few days, with lessening of the degree of cyanosis and dyspnoea and a fall in pulse rate. A blood count on the fifth day of treatment was, however, virtually unchanged. Within three weeks dyspnoea was no longer present, cyanosis was slight and the crepitations had almost completely disappeared from both lungs. The erythromycin was discontinued but the daily dose of 125 mg. of cortisone was maintained. After twenty days of treatment an open thoracotomy was performed under general anesthesia and a biopsy specimen of the lung was obtained from the right lower lobe. It was noted that the whole lung, and especially the lower lobe, felt indurated and that subpleural emphysema was present; the pericardium appeared normal. Histological examination of the specimen showed a diffuse thickening of the alveolar walls with cuboidal epithelium cells lining some of the alveoli. The thickening consisted mainly of reticulin fibres, cellular infiltration being minimal. The appearances were considered to be those of a diffuse interstitial fibrosis of the lungs consistent with that described by Hamman and Rich.

Sputums revealed mixed organisms but no tubercle bacilli. The following tests were negative and non-

TABLE II
MECHANICS OF BREATHING STUDIES

Date	Maximum Breathing Capacity		MMF*	At Rest and on Hyperventilating			Respiratory Rate	After Exercise			Respiratory Rate
				Compliance†	Resistance‡			Compliance†	Resistance‡		
	(L./min.)	% Predicted			Inspiration	Expiration			Inspiration	Expiration	
March 15, 1954 (first relapse)	89	71	....	....	....	....	..	....	....	....	..
April 25, 1955	105	84	....	.096	2.86	2.04	38	....	....	....	..
June 4, 1956 (treatment stopped)	126	101	....	.131 .081	2.81 2.61	4.79 3.03	37 79	.154	3.19	3.13	30
September 4, 1956	134	107	3.9	.083 .049	3.55 3.27	3.89 2.80	28 96	.077	3.45	4.5	28
October 2, 1956	119	95	3.44	.092 .073	3.22 3.01	4.09 3.13	24 65	.098	3.62	3.85	34
December 5, 1956 (second relapse)	112	89	3.70	.094 .076	3.39 2.31	5.14 3.29	23 73	.080	2.28	4.75	26
January 11, 1957	119	94	3.50	.048	6.92 4.13	6.21 4.67	22 53	.067	5.03	8.55	24

* Maximum midexpiratory flow.
† L./cm. H_2O pressure.
‡ cm. H_2O/L./second.

contributory: rickettsial complement fixation including Q fever, direct Coombs' test, examination for L.E. cells, thymol turbidity (3.5 units), zinc turbidity (12.4 units), thymol and cephalin cholesterol flocculation, alkaline phosphatase (12.4 King-Armstrong units), serum bilirubin (0.1 mg. per cent), Kolmer cardiolipin, blood urea (30 mg. per 100 ml.), serum albumin (2.7 gm.) and serum globulin (3.1 gm. per 100 ml.).

When the patient was discharged on the thirty-seventh hospital day she was free of symptoms except for mild exertional dyspnoea; she showed no physical signs apart from slight finger clubbing. A maintenance dose of 125 mg. daily of cortisone was prescribed.

Soon after her discharge (March, 1954) respiratory function tests were performed (Appendix and Tables I and II) the results of which were compatible with the Hamman-Rich syndrome. A uniform reduction in the subdivisions of lung volume suggested a diffuse restrictive pathologic condition; on effort a fall of 7.5 per cent in arterial oxygen saturation was recorded, a finding which in the absence of distribution defects, pulmonary oedema or arteriovenous shunts was considered to be the result of a disturbance of diffusion. An electrocardiogram showed marked improvement, in that the T wave in lead 2 became positive, that in lead 3 and AVF less deeply inverted, those in leads V2 to V6 isoelectric or positive, and the R-wave in V1 smaller. (Fig. 1B.)

Her condition remained satisfactory during the ensuing months, her dyspnoea slowly disappeared and the blood count returned to normal. The dose of cortisone was gradually reduced so that she was receiving 25 mg. daily in March, 1955, one year after her discharge from hospital. It was intended to discontinue treatment entirely but in the meantime she was allowed to go on holiday. On her return three weeks later she complained once again of marked breathlessness on exertion. Physical examination revealed a respiratory rate of 24 per minute and slight cyanosis of the tongue, although nothing abnormal was to be heard on auscultation of the chest. The electrocardiogram had deteriorated and showed a pattern closely resembling that of January 15, 1954, although the T wave inversions were somewhat less marked. (Fig. 1C.) The appearances of the chest on x-ray had not altered. Prednisone was substituted for cortisone in the dosage of 30 mg. daily for two days, 25 mg. daily for two days and 20 mg. daily thereafter, with rapid improvement in her clinical condition. After three weeks the dose was reduced to 15 mg. daily without recurrence of breathlessness or other symptoms. A leukocytosis was present on April 14, 1955 (17,900 per cu. mm.; neutrophils, 72 per cent). Respiratory function tests at this time were similar to those carried out thirteen months previously with one exception. Arterial oxygen saturation now fell 41 per cent on effort in contrast to the previous fall of 7.5 per cent. This suggested a severe diffusion defect in the lungs and was in keeping with an extension of the pathologic condition involving the alveolar walls.

The patient continued to take 15 mg. of prednisone

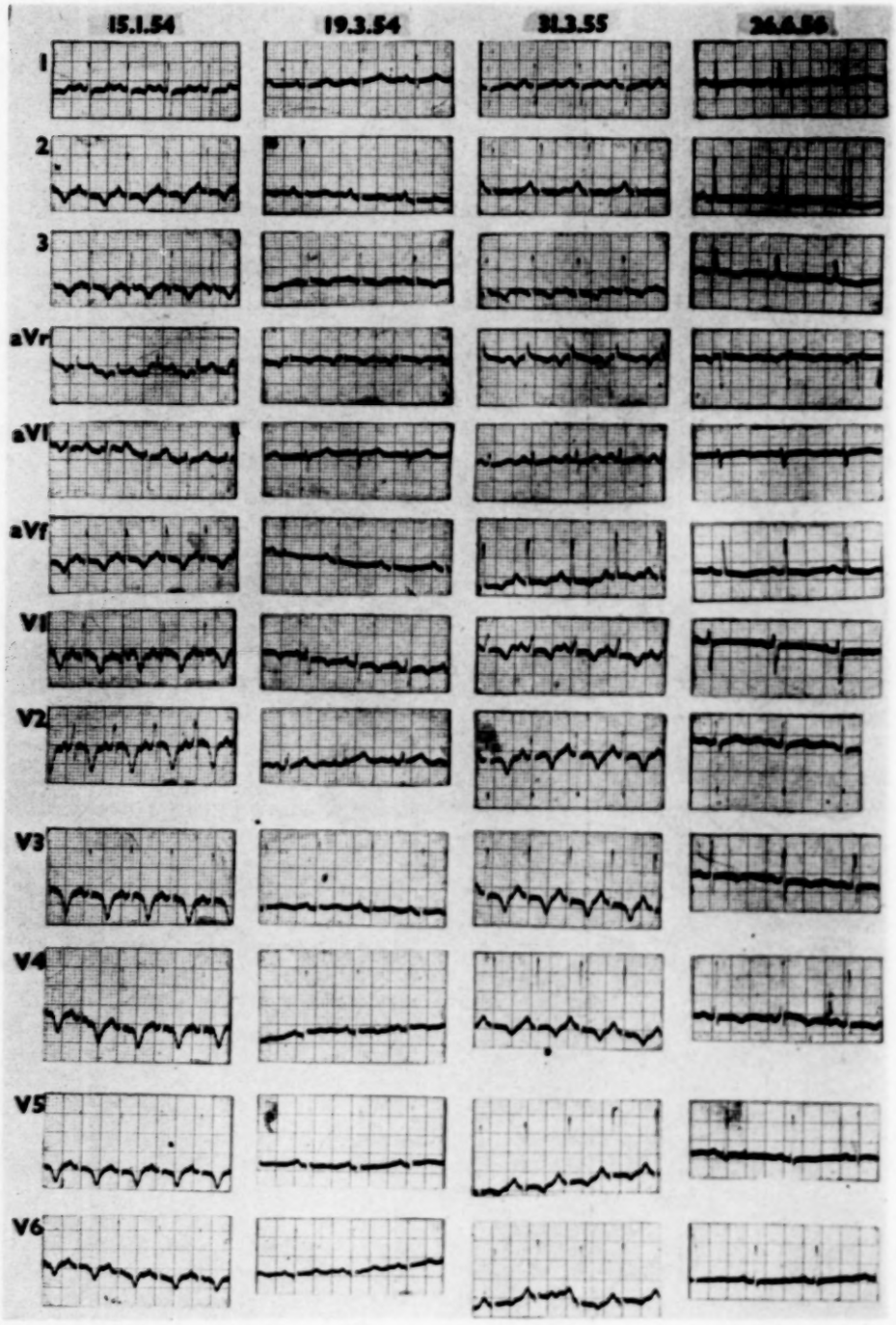


FIG. 1A–D. Electrocardiograms illustrating the variations which occurred during the course of the illness.

daily and remained well. On June 1, 1956, she was readmitted to the hospital with the object of making a second attempt at discontinuing therapy which she had by this time been receiving continuously for two years and five months. The prednisone was completely withdrawn and corticotropin was given in a dosage of 20 units subcutaneously every six hours; this was continued for three days, followed by 40 units of long-acting corticotropin on the fourth and sixth days. A leukocytosis of 28,000 per cu. mm. (69.5 per cent neutrophils) was present on the day prednisone was discontinued, a somewhat unexpected finding. Lung function tests carried out immediately after the cessation of therapy were interesting. The sub-divisions of

lung volume were closer to predicted value than on either of the previous studies; tests dependent on free airflow (maximum breathing capacity and maximal midexpiratory flow) [*12*] were normal although nonelastic respiratory resistance was slightly raised. Compliance was within normal limits and the arterial oxygen saturation fell only 5 per cent on effort. Thus all these tests showed improvement. In contrast the exercise ventilation was higher than on any previous or subsequent occasion.

The cessation of steroid therapy appeared to have no immediate adverse effect. When the patient was discharged from the hospital twenty-six days later the white cell count was 8,700 per cu. mm.; the sedimentation rate, 1 mm./hour (Westergren); the serum albumin, 3.6 gm.; and the serum globulin, 3.1 gm. per 100 cc. The electrocardiogram had improved (Fig. 1D) and resembled fairly closely that of March 19, 1954. During the ensuing seven months the patient remained extremely well and was able to live a normal life. The electrocardiogram, however, remained abnormal, showing changes identical with those of June, 1956 (Fig. 1D), and slight deterioration in serial respiratory function tests was noted. (Tables I and II.)

On January 11, 1957, the patient presented herself for examination because she had had coryzal symptoms during the previous week and had noticed slight breathlessness in the mornings. Physical examination revealed profuse crepitations throughout both lungs and the electrocardiogram had deteriorated, showing now fairly deep T wave inversions in leads 3, AVF and V1 to V6. She was readmitted to hospital. The x-ray of the chest was found to be unchanged but a leukocytosis of 14,200 per cu. mm. was present. Respiratory function tests showed no significant change since the previous studies (December, 1956) except that the oxygen saturation fell 19 per cent on effort. Prednisone was recommended in the dose of 30 mg. daily and the patient quickly improved. She was discharged ten days later at which time the fall of oxygen saturation on effort was 8 per cent. At present (April, 1957) she is maintained free of symptoms on 15 mg. of prednisone daily and physical examination is within normal limits except for clubbing of the fingers. The cardiogram has, however, not improved although the blood count has again returned to normal.

COMMENTS

The use of adrenal steroids and corticotropin has been previously reported in eleven cases of diffuse interstitial fibrosis of the lungs. Since treatment has been successful in only one other recorded instance [*3*], the manner in which it was applied is analysed in Appendix II. It is apparent that a brisk response was obtained in those patients who had a relatively short history of the disease and in whom there was evidence that the disease process was in the acute phase, *viz.* the first case of Peabody et al. [*4*], the first case of Schechter [*6*], that of Pinney and Harris [*3*] and our own. Previous writers [*3,4,7*] have also drawn attention to this, and have rightly stated that therapy, if it is to be effective, must be aggressively applied at an early stage of the disease. This is in keeping with the fact that steroids tend to inhibit the formation of inflammatory exudate and granulation tissue, but have little effect on mature scar tissue [*11*]. The case reported by Callahan et al. [*10*] was a striking and unexplained exception to this rule; unfortunately, details of cortisone therapy are not mentioned and it is possible that the dose used was inadequate.

In two cases [*4,6*] deterioration and death followed reduction in dosage or withdrawal of cortisone or corticotropin, despite reinstitution of treatment in large dosage. In our experience this "escape phenomenon" is not confined to diffuse interstitial fibrosis of the lungs. We have seen a case of acute silicosis, proved by lung biopsy, relatively well controlled on steroid therapy for fourteen months, in which a similar acute and fatal exacerbation followed withdrawal of treatment. It can be tentatively concluded, therefore, that the dose of steroid in the treatment of diffuse interstitial fibrosis of the lungs should be large and prolonged for many months. Furthermore, the relapse after a remission lasting seven months in our case demonstrates that attempts at withdrawal should be gradual, and that even when apparently successful, the patient should be carefully followed up for a long period. It has been suggested by Peabody [*4*] that steroids might lend momentum to an already downhill course. However, the experience in our case, with its repeated responses to therapy and relapses on withdrawal, indicates rather that it is inadequate dosage, or untimely withdrawal of the drug, which has been the cause of the reported fatalities. Evidently the response of chronic cases is much less satisfactory. Nevertheless, it does not seem justified to withhold treatment as long as there is some chance of halting progression of the fibrosis. An analogy can be drawn with chronic beryllium poisoning, with its similar histological appearance, in which hormone therapy is always beneficial no matter how advanced the disease [*13*].

The neutrophil leukocytosis which was present in this case is a feature which has been previously

described [*2,4,7,9*]; in one instance [*14*] it was extremely high, 82,000 per cu. mm. The fluctuations which we observed appear to have paralleled the clinical course of the disease very closely indeed. In retrospect, it seems that the high figure of 28,000 per cu. mm. which was present when the prednisone was withdrawn probably indicated activity of the disease process, despite the fact that it was not in keeping with the clinical picture. It is suggested that, when present, the leukocytosis should prove a valuable guide to the dose of steroid required.

Throughout the course of the illness the electrocardiogram showed variations which correlated well both with the clinical condition and with the state of respiratory function. It is noteworthy however that with each relapse there was not only evidence of increased right ventricular strain, but also of rather widespread ST and T wave inversions of leads on the left side of the chest. Review of the literature shows that although the typical pattern of right ventricular hypertrophy is the rule in diffuse interstitial fibrosis of the lungs, T wave inversions of the left ventricular surface leads similar to those seen in our case have been described [*2,4–7*]. These latter changes have led to the additional diagnosis of myocardial ischaemia, and this has proved correct pathologically in three elderly female patients [*4,5*]. In other cases such left ventricular T wave changes have been found at autopsy to be associated either with pericarditis [*2*] or with unexplained left ventricular hypertrophy [*4,6,7*]. It is probable that the electrocardiograms in our case represent hypertrophy of both right and left ventricles.

The main abnormality of respiratory function in diffuse interstitial fibrosis of the lungs is thought to be disturbance of diffusion [*15*]. Although accurate estimations of pulmonary diffusing capacity could not be performed in this laboratory, observations of the fall of arterial oxygen saturation during standard effort were considered to reflect this disturbance fairly closely, provided that defects of distribution, arteriovenous shunts and pulmonary oedema could be excluded, as they were in this instance. From Table I it will be seen that the most striking arterial anoxia on effort was recorded at the times of clinical relapse. Since this was the only lung function test performed which paralleled the clinical condition, we believe that it (and probably direct measurement of diffusing capacity) is the one most likely to prove of value in the assessment of therapy in this disease. Studies before and after the administration of cortisone by Cournand [*15*] in a case of sarcoidosis demonstrating the syndrome of alveolocapillary block showed similar improvement both in diffusing capacity and in the level of arterial anoxia on effort. Thus our observations are in keeping with his findings. The surprising normality of test results when the prednisone was discontinued in June, 1956, suggested that the condition had up to that time given rise to little deposition of collagen in the alveolar walls. Subsequent estimations of arterial saturation after effort showed only slight deterioration, and they were interpreted as indicating some replacement of reticulin by collagen and not a reactivation of the disease process which indeed we do not believe occurred until January 1957.

SUMMARY

A case of diffuse interstitial fibrosis of the lungs (Hamman-Rich syndrome) in a twenty-four year old woman is described.

She has been maintained in a satisfactory state of health for three years and four months so far, by the use of steroid therapy.

Respiratory function studies were performed throughout the course of the illness.

Acknowledgments: We wish to express our thanks to Mr. D. I. Adler who performed the lung biopsy, and to Dr. I. Webster for examining and commenting on the histological sections. We received much encouragement and helpful advice from Dr. Margaret Becklake, Dr. Maurice McGregor and Dr. M. M. Suzman. We are indebted to Mr. H. I. Goldman for performing the respiratory function tests.

APPENDIX I

Methods Used in Lung Function Studies. Vital capacity, expiratory reserve volume and maximum breathing capacity were measured on a light weight spirometer. Functional residual capacity was measured by the closed circuit, constant volume method of McMichael [*16*] with certain adaptations [*17*], and from the rate at which the lungs equilibrated with this circuit, a mixing index was calculated [*18,19*].

An exercise test of the type developed by Hugh-Jones [*20*] was made and the arterial saturation at rest, during this exercise, and after breathing pure oxygen, was recorded by ox-

imetry (Waters Conley Absolute Reading Oximeter).

The mechanics of breathing were studied by comparing the intraoesophageal pressure with rate of airflow in and out of the lungs. Intraoesophageal pressure was measured using a tube of the type described by Mead and Whittenberger [*21*] and a straingauge manometer (Statham P5 TC ± .7D-350) airflow using a Lilly Pneumotachygraph screen. Compliance (expressed as L./cm. H_2O pressure) was estimated by relating tidal volume to the intrathoracic pressure difference at end-inspiration and end-expiration, and resistance (expressed as cm./L./second) was calculated at the points during inspiration and expiration at which the pressure required to overcome non-elastic resistance was maximum [*21*].

Certain results were compared with the predicted normal value for the patient, namely vital capacity, residual volume and maximum breathing capacity [*22*]. The prediction formulas used were based on a study of ninety-four normal subjects of varying ages, resident at this altitude (5,670 feet above sea level) in which test results were correlated with four body characteristics (namely age, height, weight and surface area) and formulas for prediction were based on one or more of these variables, by standard statistical technic of partial correlation.

APPENDIX II

Analysis of Steroid Therapy in Previously Reported Cases of Diffuse Interstitial Fibrosis of the Lungs.

1. Cox and Kohl [*8*] report the use of "small doses of ACTH" in a fifty-nine year old woman who had had symptoms for three years. There was no response to therapy and the patient died five weeks after admission.

2. Silverman and Talbot [*9*] used 200 to 250 mg. of cortisone daily for three weeks in a man of thirty-six whose symptoms had been present for sixteen months. The dose was gradually reduced to 75 mg. at the end of two months. There was moderate improvement at first but deterioration and death followed six weeks after cessation of therapy.

3. Callahan et al. [*10*] observed no noticeable effect from therapy in a fifty-five year old man ill for two months. The patient died twenty-seven days after admission. Treatment consisted of 25 units of ACTH daily, increasing later to 75 units every six hours, together with an unstated dose of cortisone.

4. The first case of Peabody et al. [*4*], a thirty-one year old man whose symptoms had been present for three months, responded rapidly to 25 units of ACTH daily. The dose was reduced to 10 units, and after a brief course of ACTH-gel cortisone, was used in the dose of 100 mg. daily with gradual reduction over one month, with further ACTH in small dosage at the end of therapy. Sudden death occurred five days after treatment was stopped despite reinstitution of ACTH and cortisone in massive dosage.

5. The second case of Peabody et al. [*4*], a woman of seventy-two years, ill for two years, responded slightly to 75 mg. of cortisone daily, but sudden death occurred two weeks after treatment was discontinued.

6. The third case of Peabody et al. [*4*] a thirty-eight year old man ill for eight years received varying doses of ACTH for thirty-two days with subjective improvement initially. Death occurred eleven days after reduction of dosage despite administration of 50 units of ACTH and 300 mg. of cortisone daily.

7. Schechter's first case [*6*], a thirty-one year old man ill for three months, showed marked clinical and radiological improvement on 25 units of ACTH daily for three weeks followed by 200 mg. of cortisone daily for two months, the cortisone being gradually reduced over one and one-half weeks. However, deterioration and death followed four days after the cortisone was discontinued despite the administration of 30 units of ACTH and 800 mg. of cortisone.

8. Schechter's second case [*6*], a thirty-eight year old man ill for five years, failed to respond to an unstated dose of ACTH for thirty days, and death occurred while he was still receiving treatment.

9. A fifty-two year old man, ill for five years, was treated with 60 units of ACTH daily for five weeks by Wildberger and Barclay [*7*]. Although he was symptomatically better at first he died while still being treated.

10. Wildberger and Barclay's second case, a thirty-eight year old man ill for eight years, had an exacerbation of symptoms four months prior to admission. He improved on an unstated dose of ACTH for four weeks but death occurred when the drug was gradually withdrawn, despite the administration of high doses of ACTH and cortisone.

11. A twenty-seven year old woman, ill for six months, was treated with cortisone by Pinney and Harris [*3*]. She showed marked improve-

ment in forty-eight hours and remains well at the end of seventeen months. The dose was as follows: 200 mg. of cortisone for twenty-three days; 150 mg. for one day; 100 mg. for forty-one days; 75 mg. for three months; 50 mg. for eight months; and 40 mg. daily till the present.

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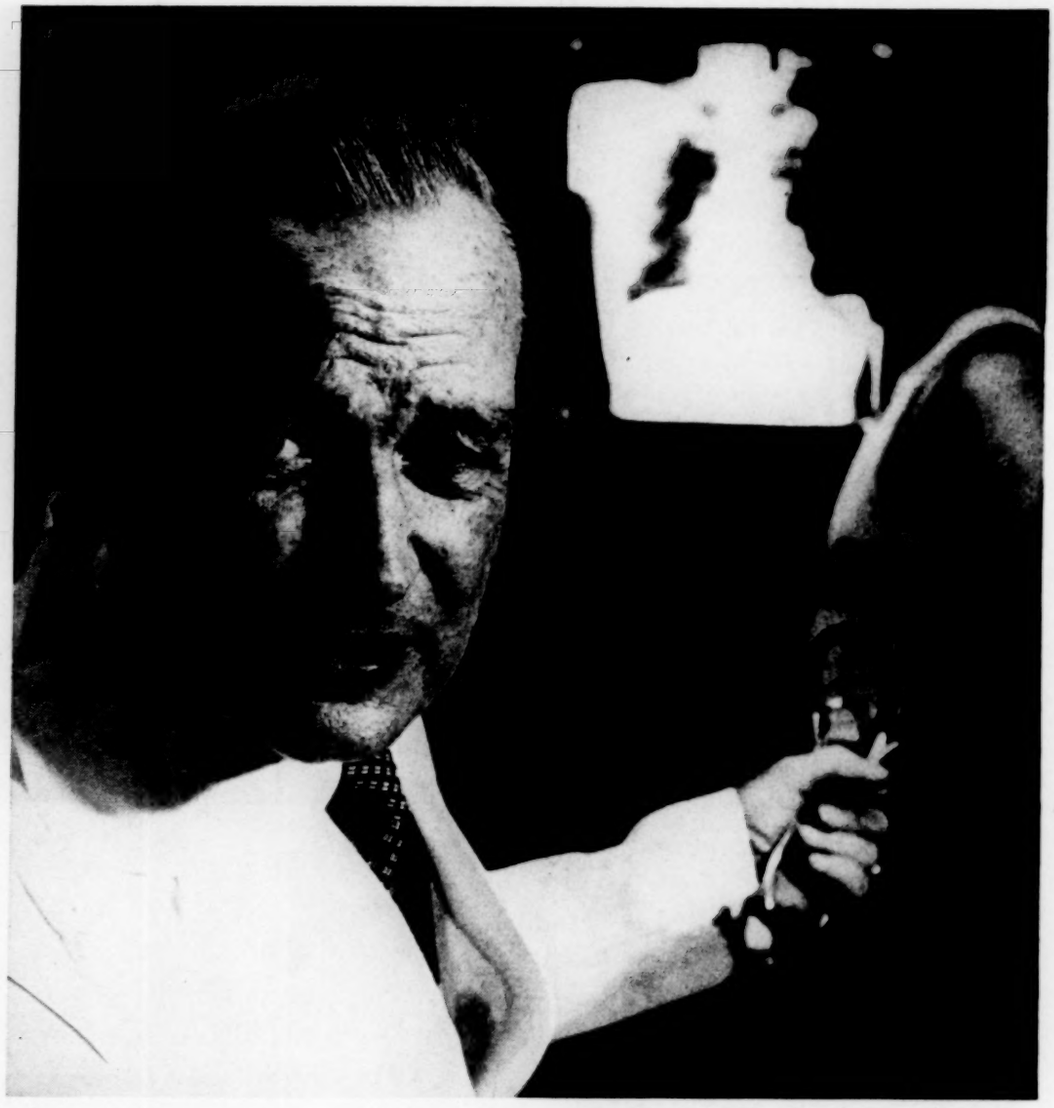
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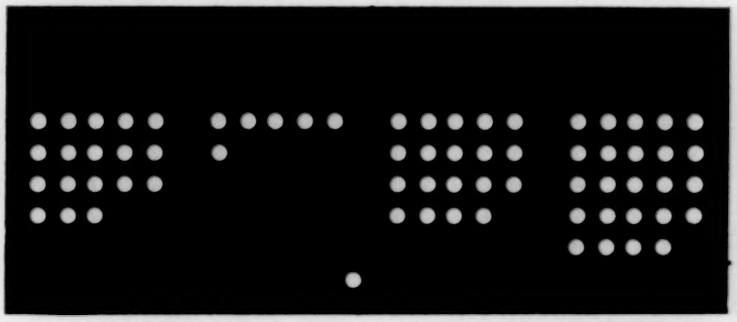
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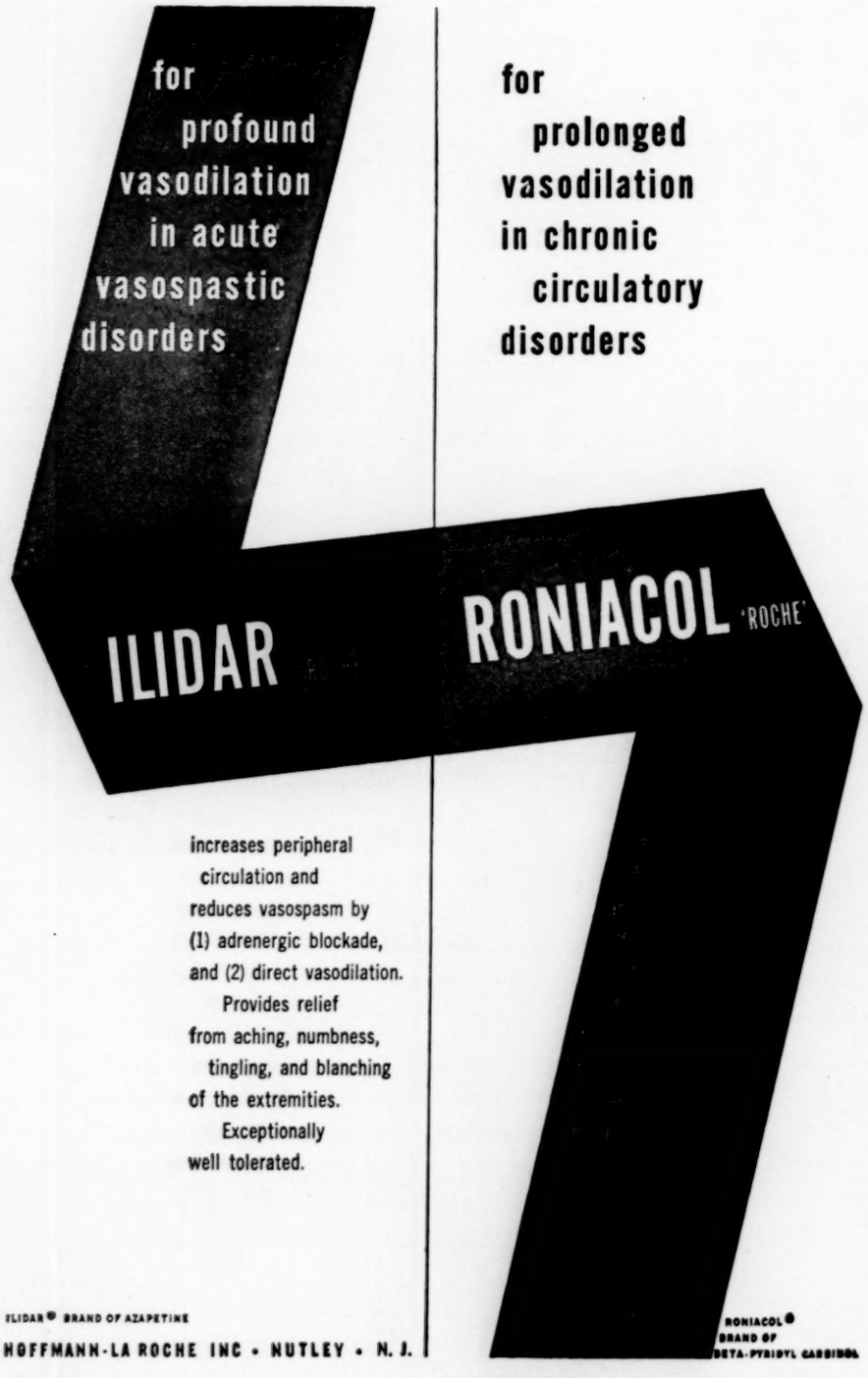
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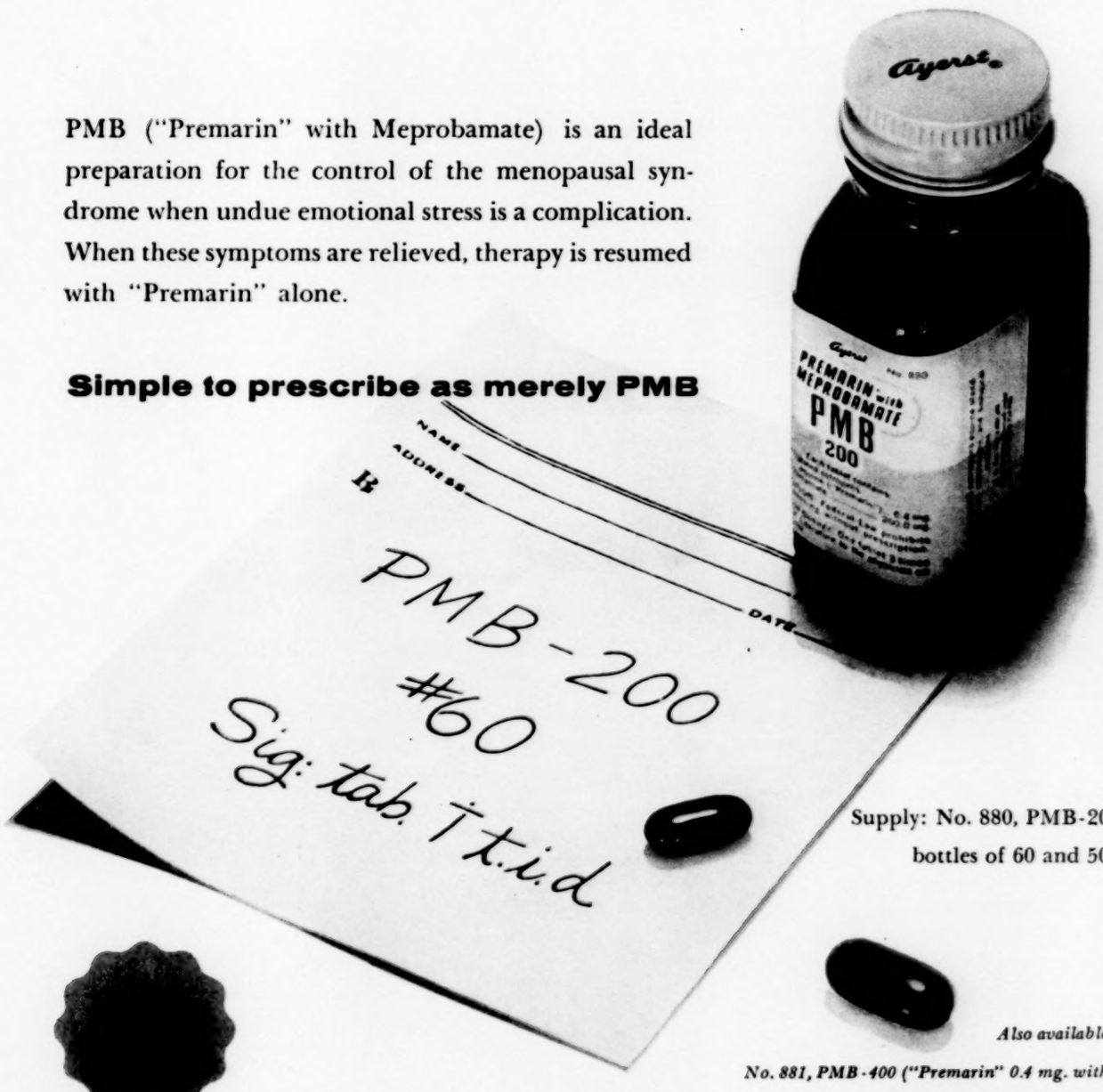
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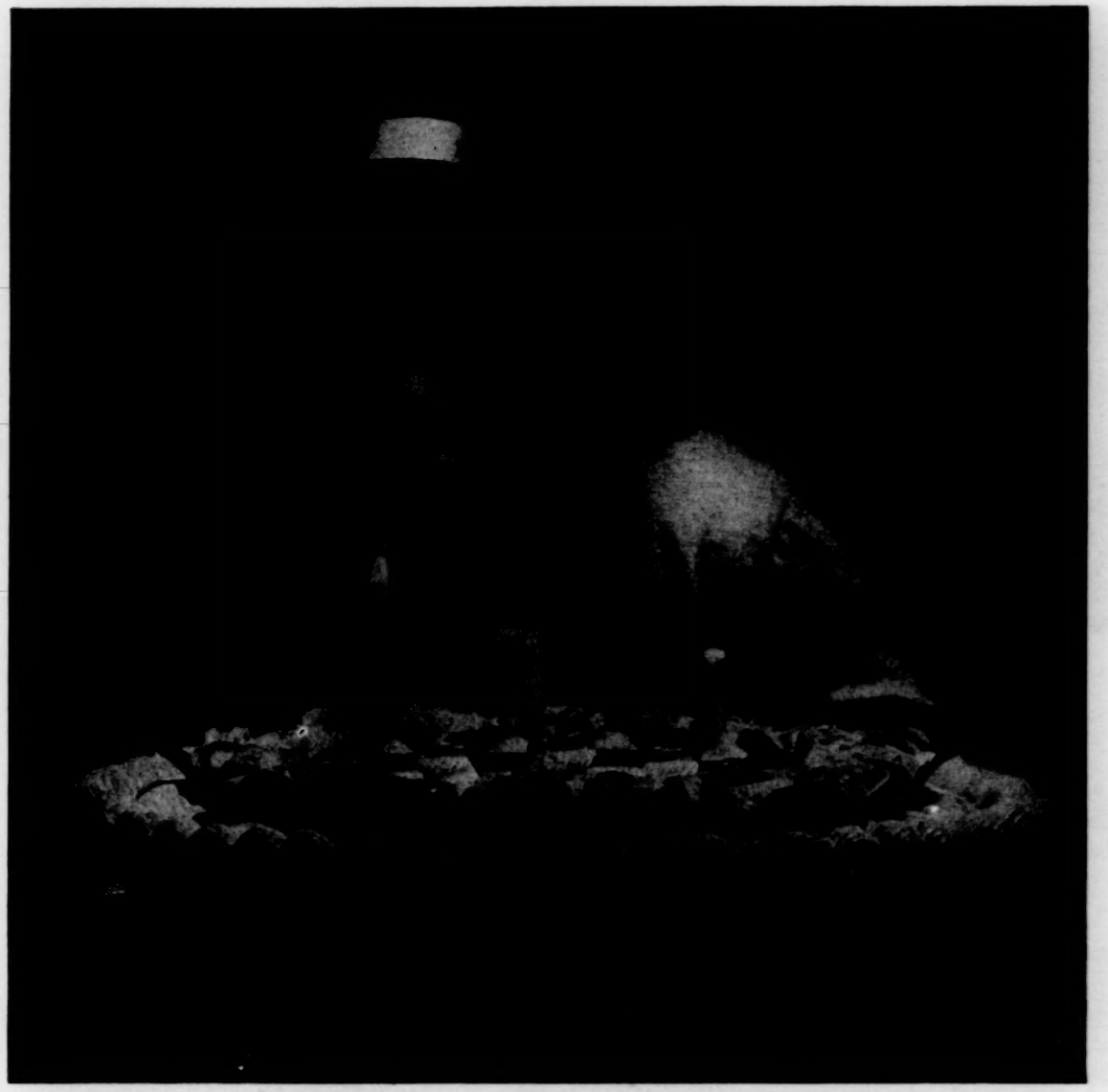
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no asthma symptoms

...thanks to Tedral prescribed by her physician.

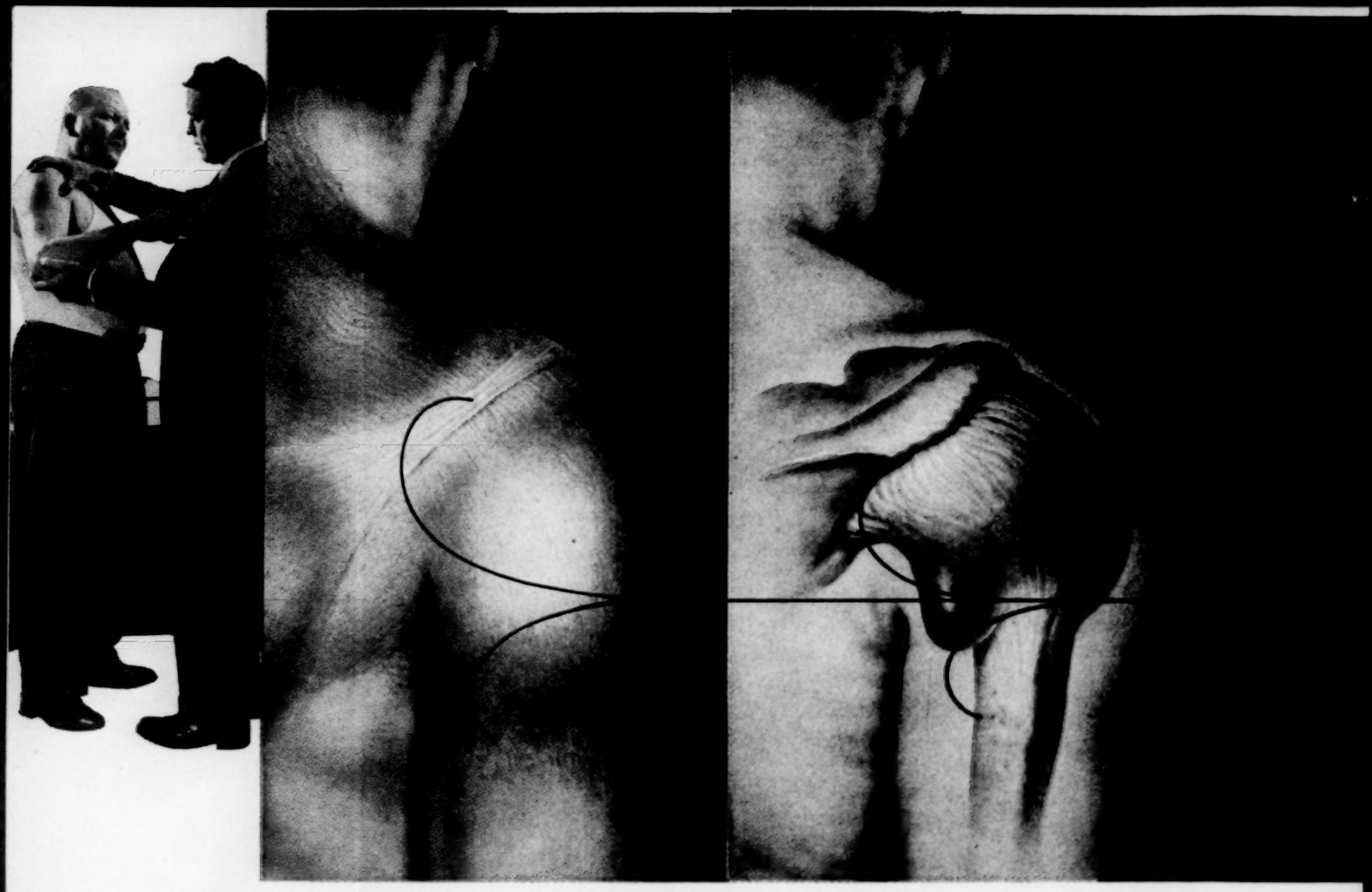
No single drug can equal Tedral to protect

the asthmatic patient against symptoms' round the clock.

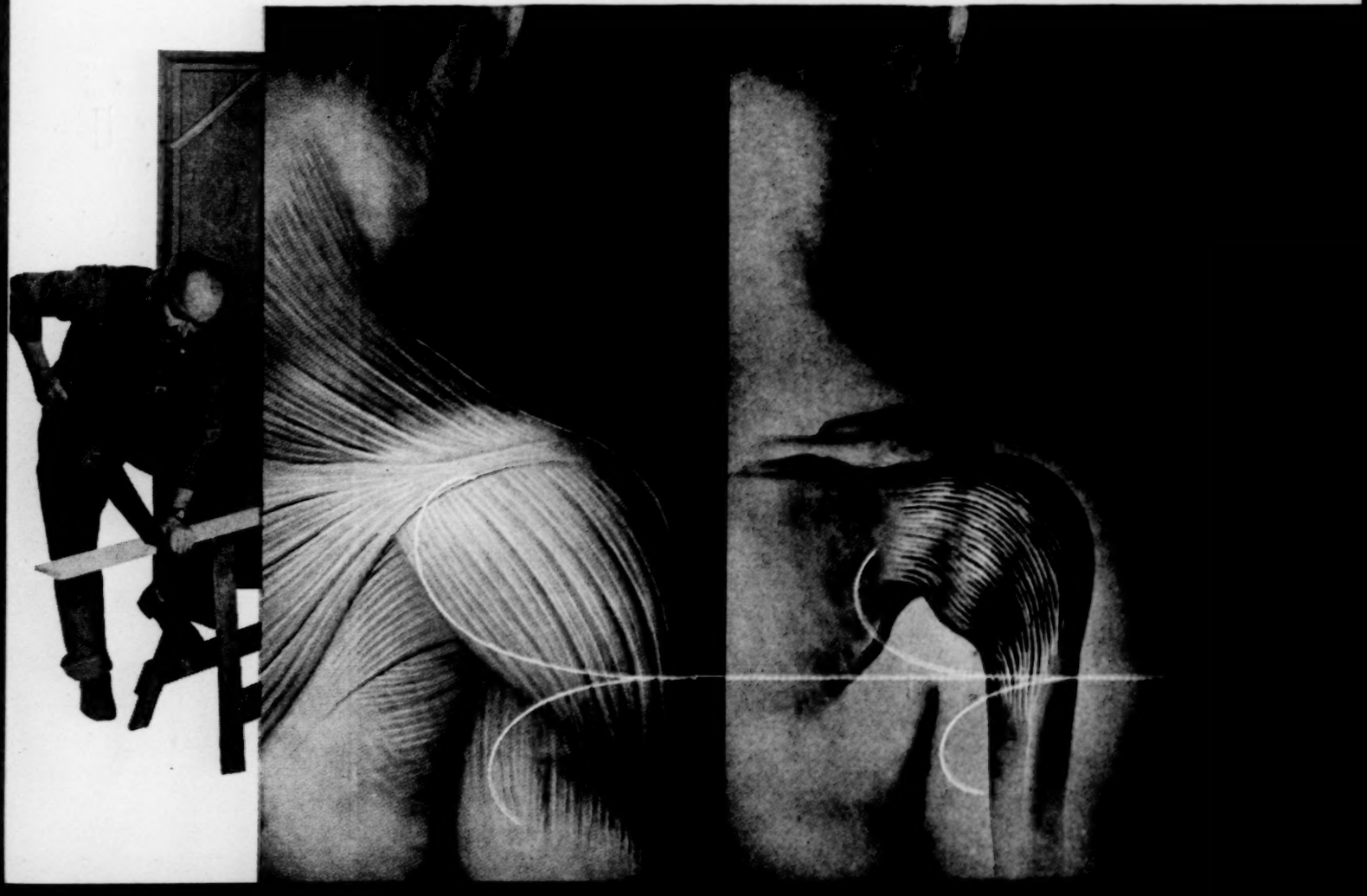
Dosage: 1 or 2 tablets q.i.d. Available: boxes of 24, 120 and 1,000.

Tedral®

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"Rheumatoid arthritis is a constitutional disease with symptoms affecting chiefly joints and muscles."[1] "Pain in the affected joint is accompanied by splinting of the adiacent muscles, with resultant 'muscle spasm.' "[2]



rheumatoid arthritis involves both joints and muscles only

MEPROLONE is the only anti-rheumatic-antiarthritic designed to relieve simultaneously (a) muscle spasm (b) joint-muscle inflammation (c) physical distress . . . and may thereby help prevent deformity and disability in more arthritic patients to a greater degree than ever before.

SUPPLIED: Multiple Compressed Tablets in bottles of 100, in three formulas:
MEPROLONE-5—5.0 mg. prednisolone, 400 mg. meprobamate and 200 mg. dried aluminum hydroxide gel.
MEPROLONE-2—2.0 mg. prednisolone, 200 mg. meprobamate and 200 mg. dried aluminum hydroxide gel.
MEPROLONE-1—supplies 1.0 mg. prednisolone in the same formula as MEPROLONE-2.

1. Comroe's Arthritis: Hollander, J. L., p. 149 (Fifth Edition, Lea & Febiger, Philadelphia, Pa. 1953).
2. Merck Manual: Lyght, C. E., p. 1102 (Ninth Edition, Merck & Co., Inc., Rahway, N. J. 1956).

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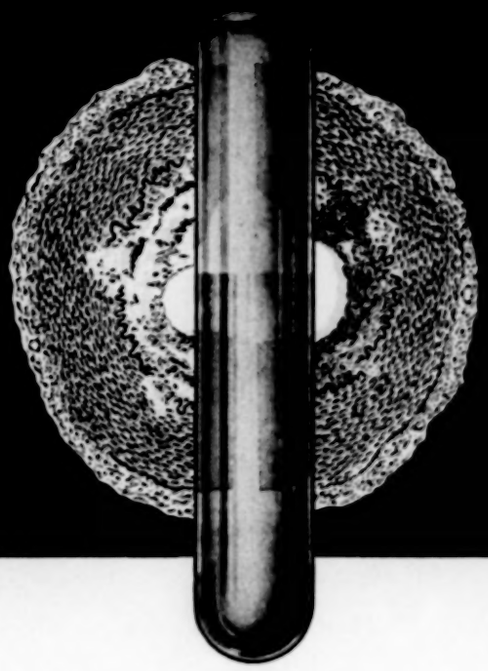
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MODERN DRUGS

IN DIABETES

Blood Cholesterol Regulation Is Desirable



Frequently, diabetes mellitus is associated with excessive coronary atherosclerosis and heart disease. It is also well recognized that vascular disease manifests itself at an earlier age and progresses more rapidly in the diabetic . . . especially the diabetic female. The early onset and severe degree of atherosclerosis may be due to faulty lipid metabolism, with a concomitant elevation in blood cholesterol levels. Kinsell[1] reports that even in diabetics with extensive vascular disease it is possible to produce normal cholesterol levels with an essential fatty acid emulsion.

The consensus of opinion today is that elevated cholesterol levels should be reduced or prevented. This can be done very well by the addition of adequate amounts of linoleic acid and vitamin B_6 to the diet. In scores of diabetic patients, with extensive vascular disease, diets high in linoleic acid resulted in improvement.[2,3] Vitamin B_6 apparently is necessary to convert linoleic acid into the primary essential fatty acid, arachidonic acid. Thus the body is dependent on an intake of both linoleic acid and vitamin B_6 for normal cholesterol levels.[4,5]

This is why ARCOFAC (Armour Cholesterol Lowering Factor) provides *both* linoleic acid and vitamin B_6 in adequate amounts, thus allowing the patient to eat a balanced, nutritious and palatable diet. Prophylactic dosage is 1-2 tablespoonfuls daily; therapeutic—2-8 tablespoonfuls daily.

Each tablespoonful (15 ml.) of Arcofac emulsion contains:

Essential fatty acids†.....6.8 Gm.
(measured as linoleic) with 2.5 I.U. of Vitamin E*

Pyridoxine hydrochloride
(Vitamin B_6).....1.0 mg.

†Supplied by safflower oil which contains the highest concentration of polyunsaturated fatty acids of any commercially available vegetable oil.

*Added as Mixed Tocopherols Concentrate, N.F.

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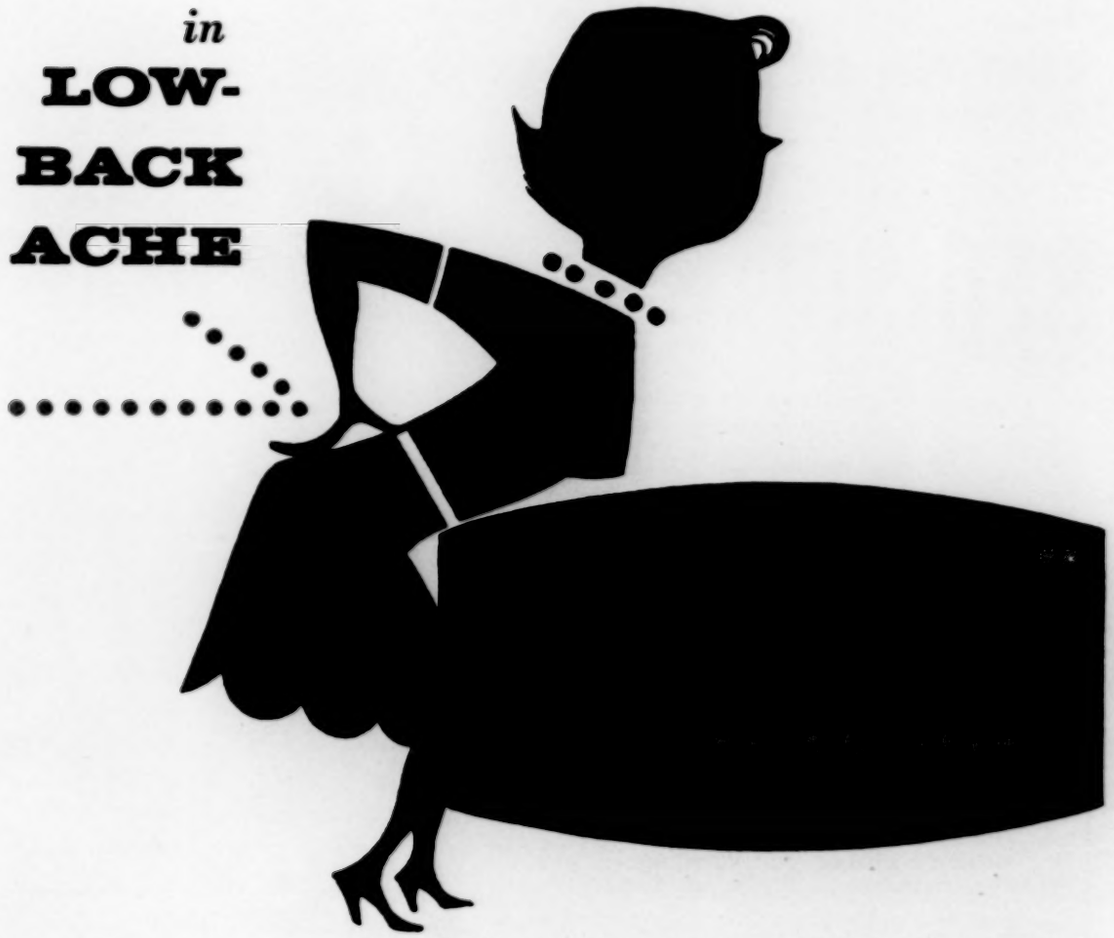
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Armour Cholesterol Lowering Factor

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A DIVISION OF ARMOUR AND COMPANY • KANKAKEE, ILLINOIS

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SPASM
PAIN
DEPRESSION

in
LOW-BACK ACHE



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Dosage: 1 tablet (50 mg.) t.i.d.

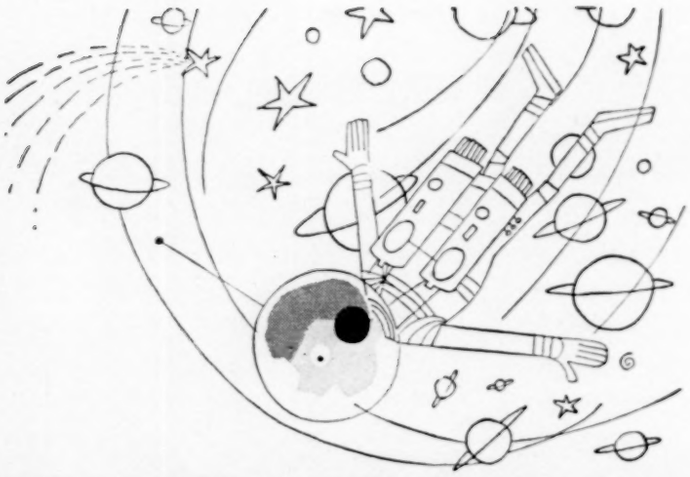
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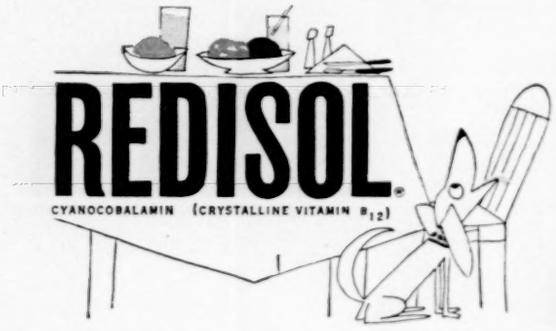
*Trademark of Brocades-Stheeman & Pharmacia. U.S. Patent No. 2,567,351. Other patents pending.

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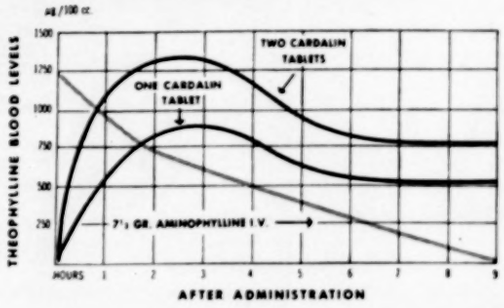
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LEVONOR...the appetite suppressant that can be given as late as 8 p.m.



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In a study[1] of its effects on 173 overweight patients, "none of the patients complained of loss of sleep. In fact when 5 mg. of LEVONOR was given at 8 p.m. no interference with sleep was noted; night hunger was markedly diminished." This is a unique advantage since it is in the evening when most obese patients are tempted to break their diet.

no CNS overstimulation

LEVONOR has no effect on the mood of the patient. It does not overstimulate the cerebral cortex, thus avoiding jitteriness, tenseness, nervousness and disturbance of sleep.

depression of appetite is its primary effect

Unlike d-amphetamine, LEVONOR is *not* a central nervous system stimulant—its primary effect is to depress the appetite. Impressive results, even with late evening doses, are obtained without the addition of sedatives.[1-5]

five times safer than dextro-amphetamine

LEVONOR's much greater safety (LD/50) and, concomitantly, its far greater freedom from side effects have been striking findings in extensive toxicity studies.[1]

here are typical clinical results with LEVONOR:

STUDY NO. I[1]

Number of patients	173
Average daily dose	2-3 tablets (5 mg. each)
Average duration of treatment	6 weeks
Average weekly weight loss	2-2½ lbs.
Side effects	9*

*Minimized by dosage adjustment

STUDY NO. II[2]

Number of patients	52
Average daily dose	2-3 tablets (5 mg. each)
Average duration of treatment	9 weeks
Average weekly weight loss	2.1 lbs.
Side effects	1*

*Adjusted with dosage

economy and low dosage of LEVONOR make it possible to administer this drug long enough to favorably alter the patient's eating habits.

Administration and Dosage:

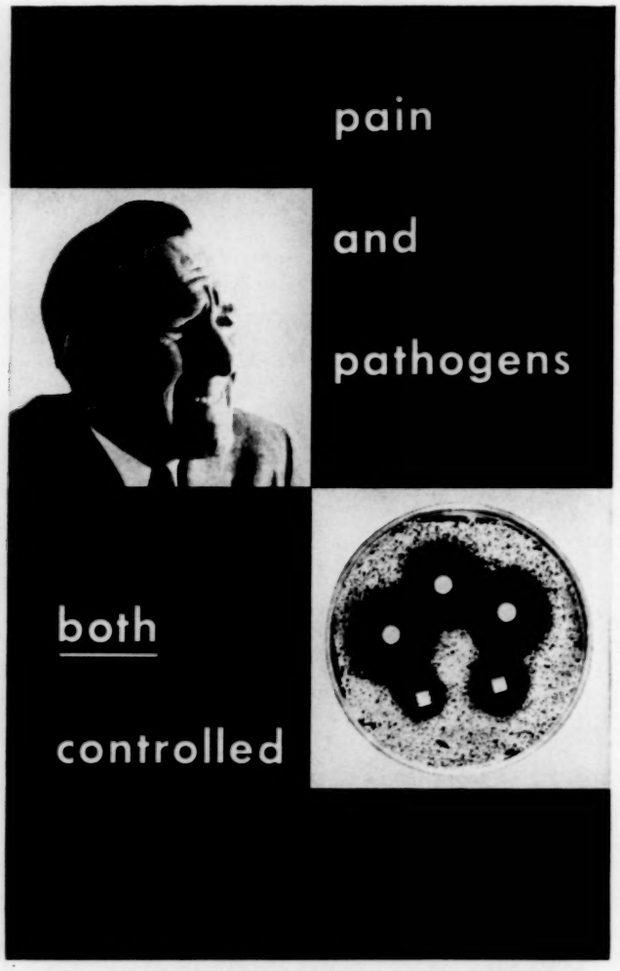
Average dose: 5 to 10 mg. twice daily.

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1. Sc. Exhibit, N. Y. State Med. Meeting, Feb. 18-21, 1957.
2. Pomeränze, J.: Report 807: 1957.
3. Frohman, I. P.: Report 315: 1957.
4. Dwyer, Thomas: Report 912: 1957.
5. Gadek, R. J.: Report 186: 1957.
6. Sc. Exhibit, Mich. State Med. Soc. Meeting Sept. 25-27, 1957.

†Patent Pending °Trademark

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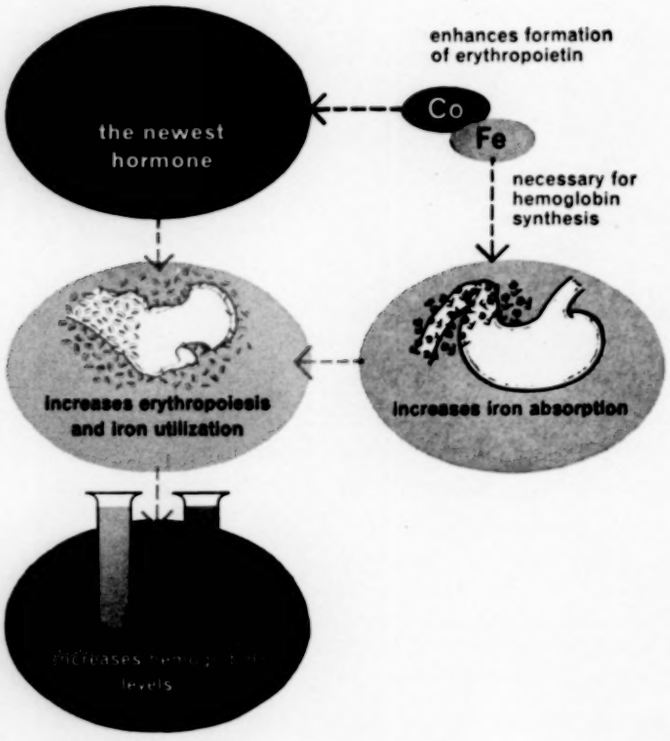


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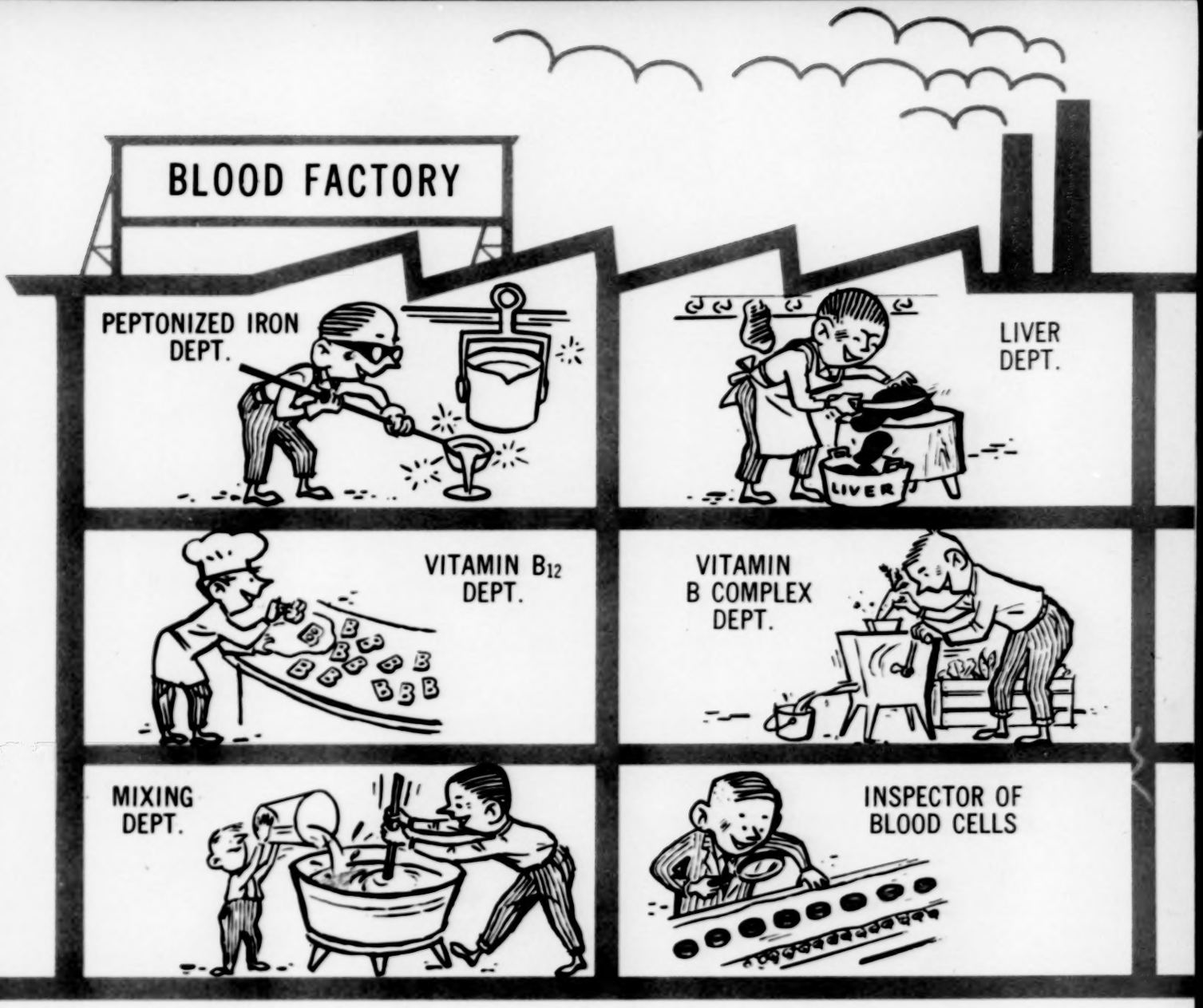
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Pantothenic acid	5 mg.
Liver fraction 1	2 Gm.
Rice bran extract	1 Gm.
Inositol	30 mg.
Choline	60 mg.

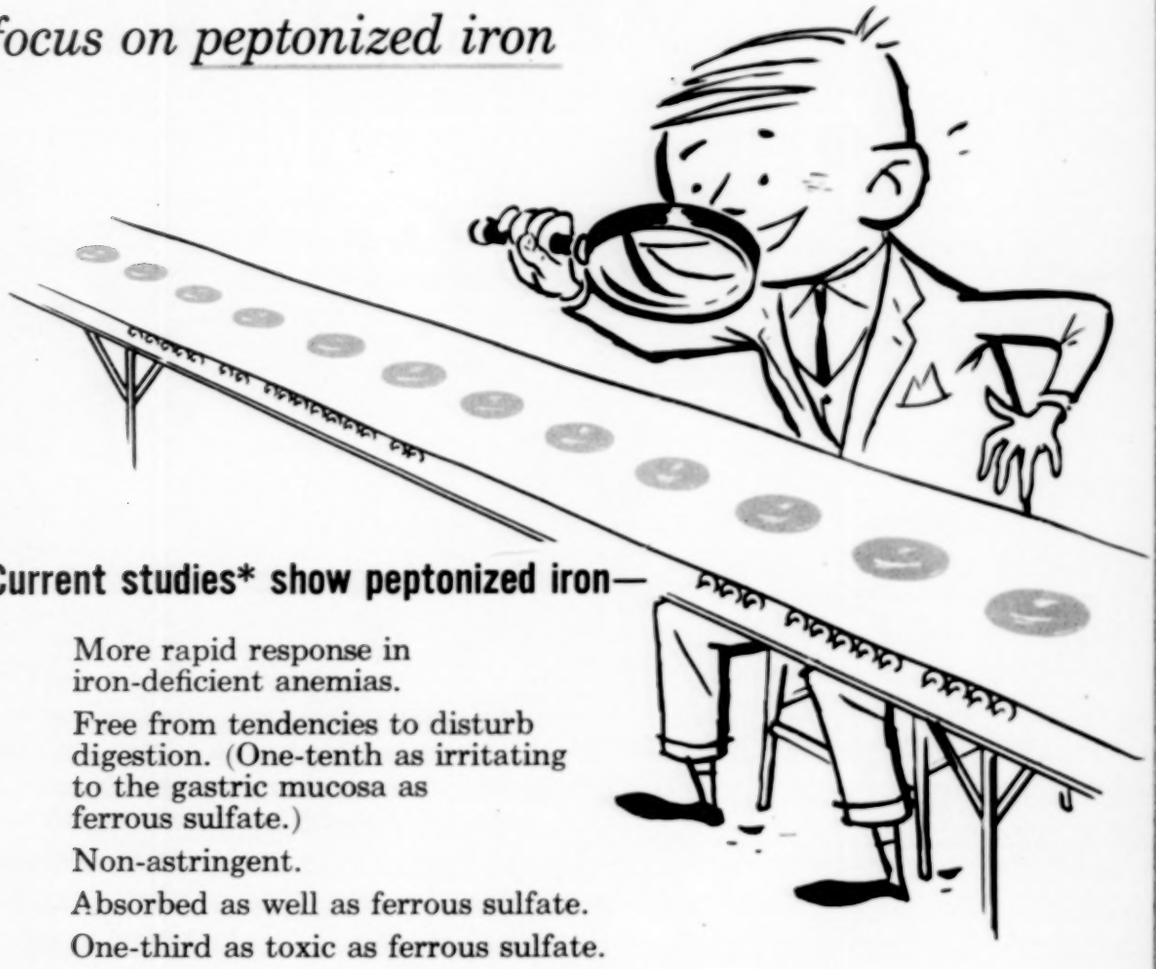
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*Keith, J.H.: Utilization and Toxicity of Peptonized Iron and Ferrous Sulfate, Am. J. Clin. Nutrition *1*:35 (Jan.-Feb., 1957).

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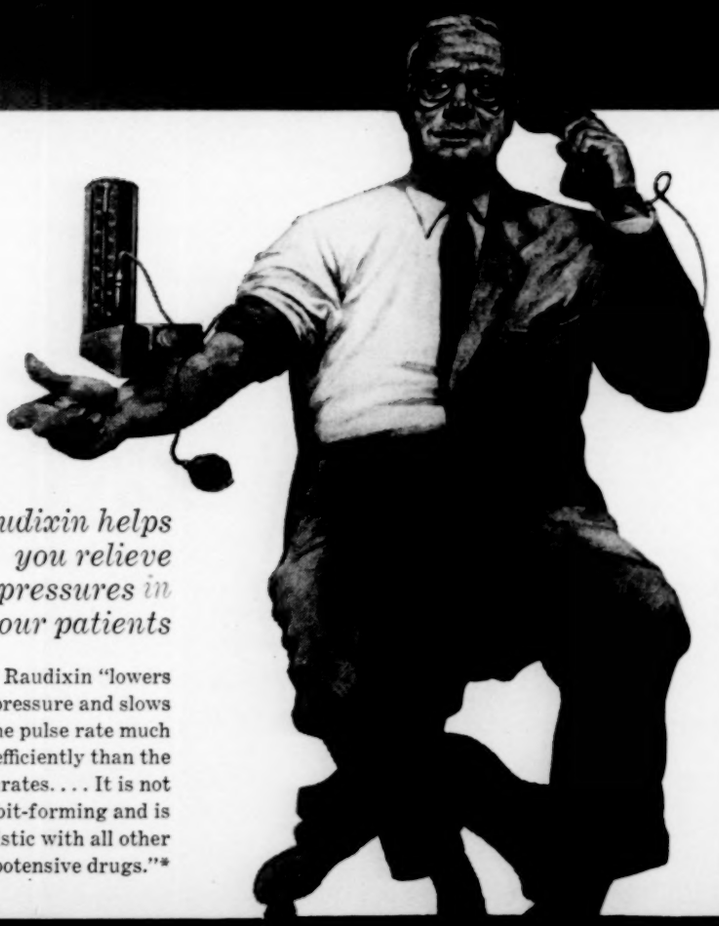
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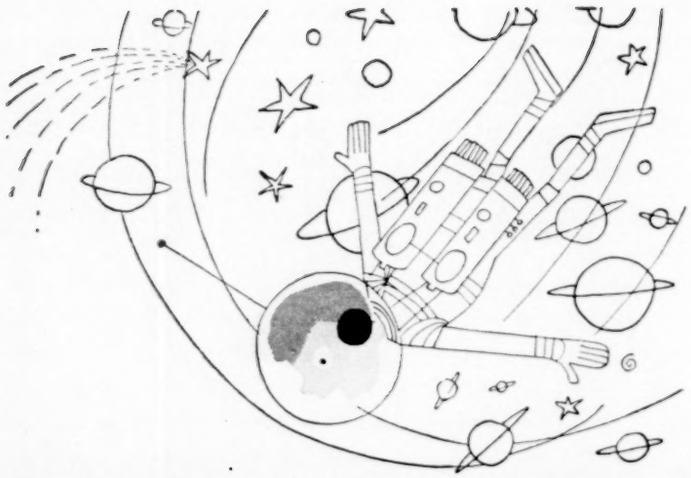
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SUPPLIED—Tablets, scored, orange, bottles of 50. Each tablet contains 250 mg. of PARAFLEX.

REFERENCES—(1) Settel, E.: Personal communication. (2) Holley, H. L.: Personal communication. (3) Burns, J. J.; Trousof, N., and Brodie, B. B.: To be published. (4) Smith, R. T.: To be published. (5) Peak, W. P., and Smith, R. T.: To be published. (6) Wiesel, L. L.: Personal communication. (7) Passarelli, W. W.: Personal communication.

CLINICAL RESULTS WITH PARAFLEX

Investigator	Disorder	Number of patients treated	Number of patients benefited	Comment
Settel[1]	acute low back pain, acute traumatic myofascitis, or osteoarthritis	15	14	response excellent in nine, good in five
Holley[2]	wry neck, cervical spondylitis, and disc syndrome	10	10	improvement, ranging from some amelioration of symptoms to profound relief
Wiesel[6]	advanced osteoarthritis	12	10	less muscle spasm and pain
Passarelli[7]	degenerative and rheumatoid arthritis	9	9	improvement, with less stiffness and freer motion
Passarelli[7]	varied arthritic rheumatic, and traumatic disorders	6	6	less stiffness, less pain
Totals		52	49	

*Trade-mark †U. S. Patent Pending

174A88

When "Breakfast Anorexia" must be Overcome

Anorexia upon arising must often be dealt with as part of a therapeutic regimen. In addition to the well-known syndrome of early pregnancy, several other causes of "breakfast anorexia" are recognized. Generally psychological in character, they include fear or lack of enthusiasm in facing the day, distaste or intolerance for the fried and fatty foods commonly served at breakfast, and habit patterns such as late rising with the inevitable morning rush. Obese patients who "cannot" eat breakfast tend to overeat during the day in order to overcome the deficit.

When "breakfast anorexia" must be overcome, physicians find that a dish of steaming oatmeal as the main food at breakfast is a great help in the solution of this problem. Its inviting warmth and delicious taste make the oatmeal dish appealing even when appetite is poorest. It also provides readily available energy for a morning of productive work; it helps to allay hunger throughout the morning; it makes a notable contribution to the day's nutritional needs; it is low in fat, and is easily digested.

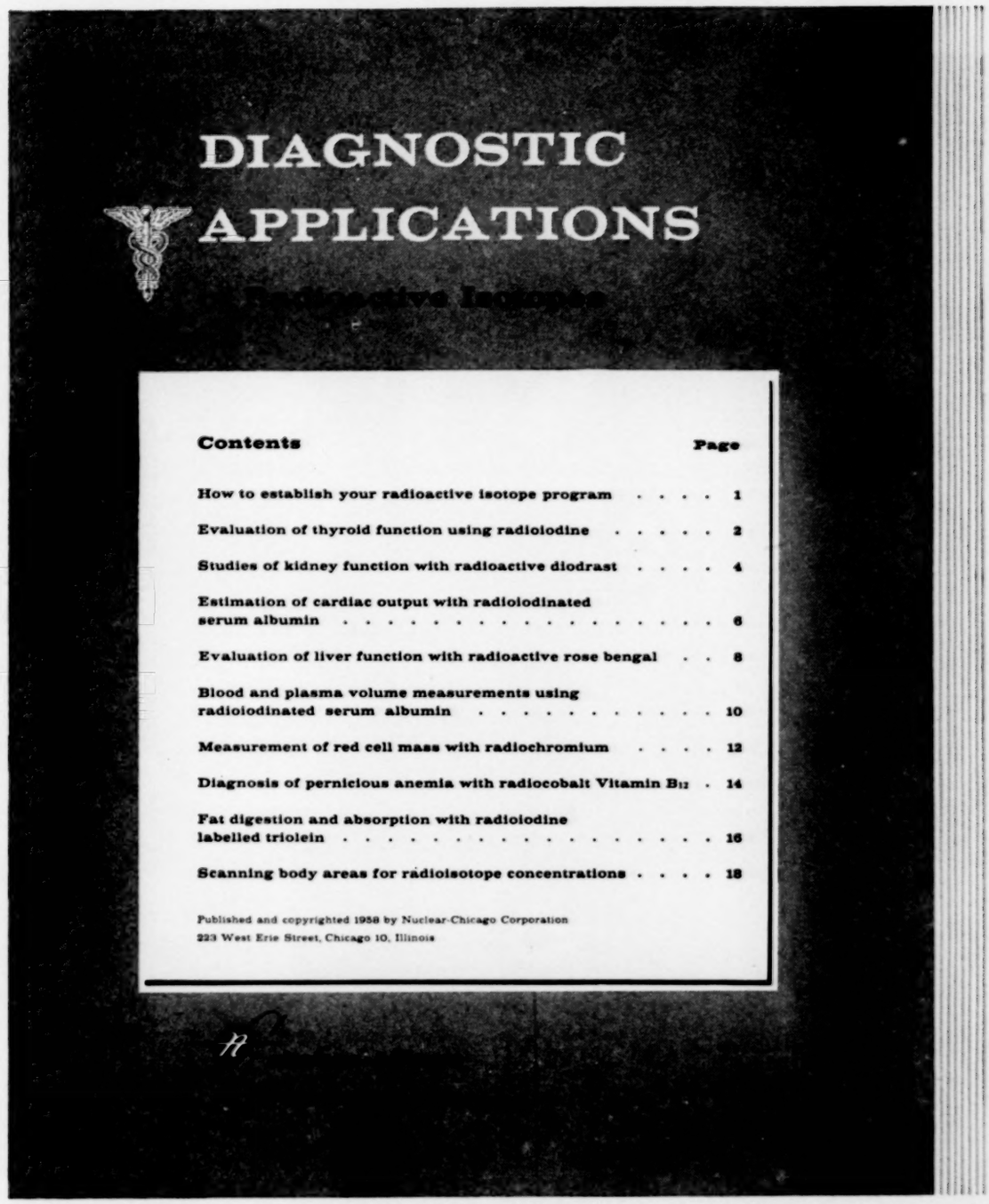
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1. M. Clin. North America 38:485 (March) 1954. 2. J.A.M.A. 162:1031, 1956. 3. J.A.M.A. 156:680, 1954. 4. Yale J. Biol. & Med. 28:308, 1955/56.

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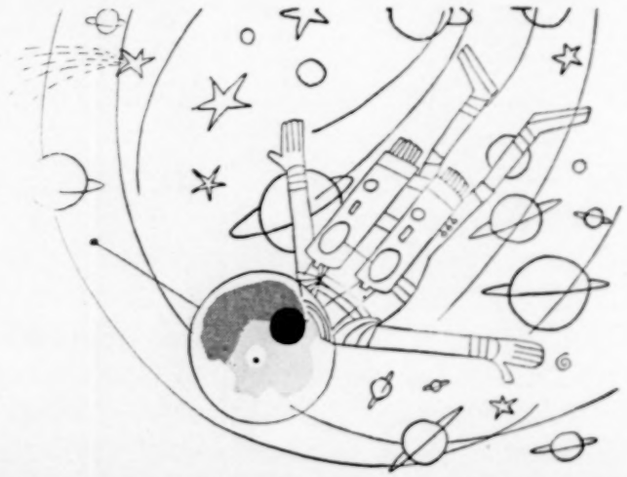
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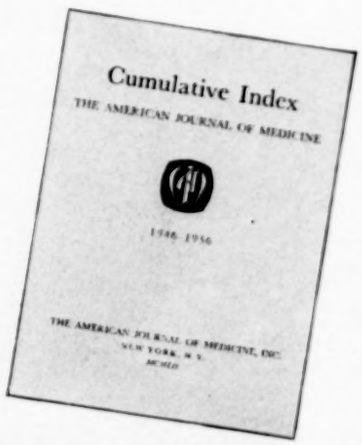
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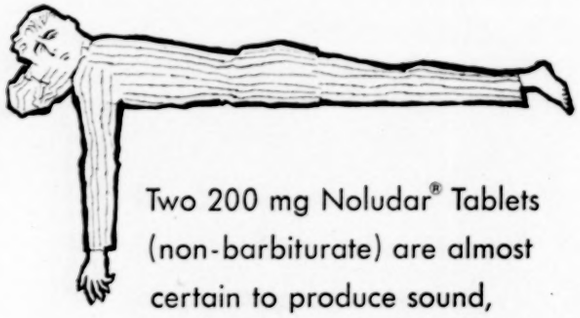
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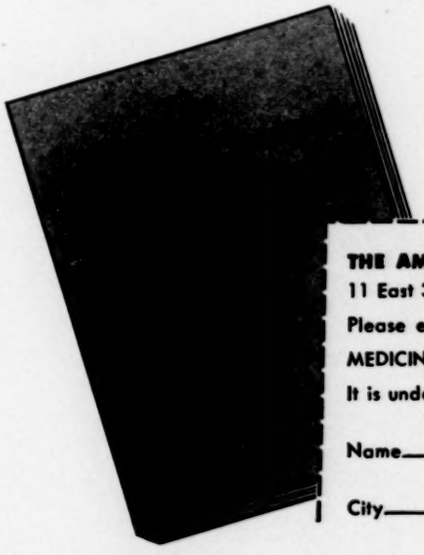
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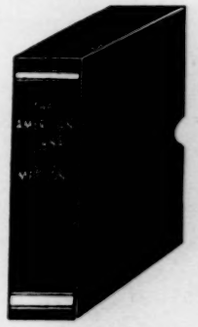
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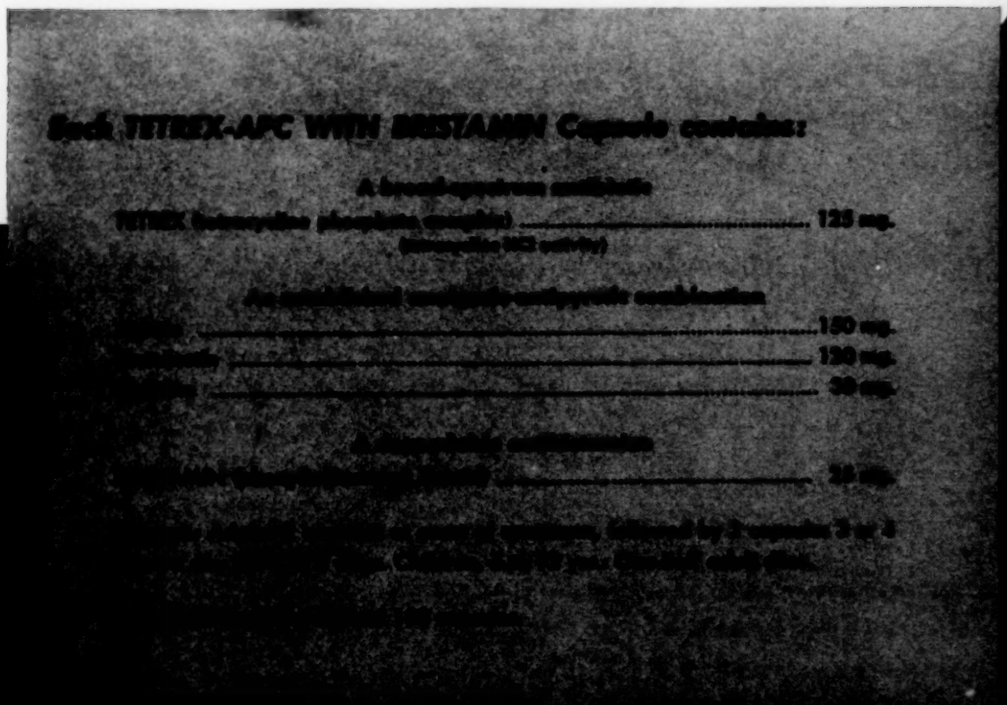
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References: 1. Flodin, N. W.: Am. Miller & Processor *81*:30 (July) 1953. 2. Block, R. J., in Advances in Protein Chemistry, Anson, M. L., and Edsall, J. T., eds., New York, Academic Press, Inc., 1945, vol. 2, p. 119.

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Council on Pharmacy and Chemistry, New and Nonofficial Remedies: Mecamylamine Hydrochloride, J.A.M.A. 162: 1469-1471, Dec. 15, 1956.

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a greatly improved ganglionic blocking agent

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- of the orally effective blocking agents, only 'Inversine' is completely and uniformly absorbed
- it provides predictable, reproducible effects with minimal day-to-day fluctuations in blood pressure response
- 'Inversine' is effective in low dosage
- permits convenient dosage schedules
- usefulness not limited by development of tolerance
- it has a gradual onset of effect, reducing the likelihood of sudden drops in blood pressure

'Diuril'

CHLOROTHIAZIDE

new and unique antihypertensive agent

- provides basic therapy to improve and simplify the management of hypertension
- often reduces dosage requirement of ganglionic blocking agents and other antihypertensive agents below the level of serious side effects
- added to other antihypertensive agents, is often effective in controlling blood pressure of even highly resistant cases
- smooths out blood pressure fluctuations
- effectiveness not diminished by development of tolerance
- well tolerated even at maximum therapeutic doses

DOSAGE RECOMMENDATIONS

New Patients

1. *Initiate 'Diuril' therapy*

'Diuril' is given in a dosage range of from 250 mg. twice a day to 500 mg. three times a day, depending on severity of the hypertension.

2. *Add 'Inversine' as follows:*

('Inversine' is established in the same manner whether used with 'Diuril' or alone.) Recommended initial dosage is 2.5 mg. 'Inversine' twice a day, preferably after meals. May be increased by 2.5 mg. at intervals of no less than two days until desired response is obtained. In severe or urgent cases, the increments may have to be larger or more frequent, with the largest dose given preferably at noon or in the evening. 'Inversine' is extremely potent and should always be titrated according to the patient's orthostatic blood pressure response.

3. *Adjust dosage of 'Diuril' for optimal response.*

Patients on 'Inversine' and/or other ganglionic blocking agents

1. *Initiate 'Diuril' therapy*

'Diuril' is given in a dosage range of from 250 mg. twice a day to 500 mg. three times a day, depending on severity of the hypertension.

2. *Adjust dosage of ganglionic blocking agent*

If the patient is established on a ganglionic blocking agent (e.g., 'Inversine') it should be continued, but the total daily dosage should *immediately* be reduced by as much as 25 to 50 per cent. This will reduce the serious side effects often observed with ganglionic blockade.

If other antihypertensive agents are used, their dosage should be adjusted as indicated by patient response.

3. *Determine optimal maintenance dosage*

The patient *must* be observed frequently and careful adjustment of all agents should be made to determine optimal maintenance dosage.

PRECAUTIONS: Side effects of 'Inversine' are essentially the same as those encountered with other ganglionic blocking agents. At the first sign of constipation, vigorous treatment must be initiated immediately since paralytic ileus may result if constipation is unchecked. Patients should be informed how to cope with postural hypotension should this occur. 'Inversine' is contraindicated in coronary insufficiency, organic pyloric stenosis and recent myocardial infarction.

SUPPLIED: 'Inversine,' tablets of 2.5 mg. and 10 mg. Bottles of 100. 'Diuril,' tablets of 250 mg. and 500 mg. Bottles of 100 and 1000.

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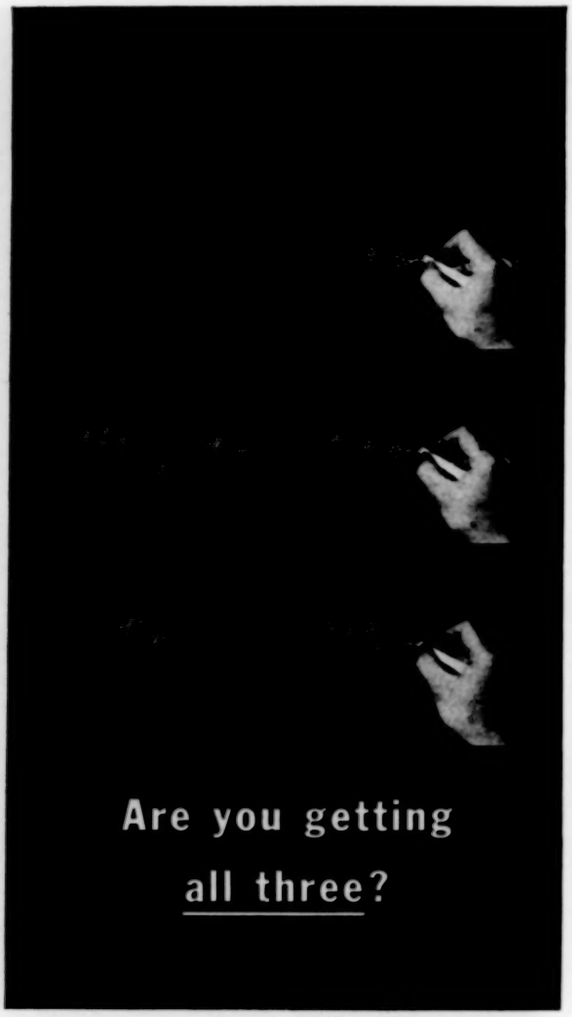
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1. Wright, W. T. Jr.; Pokorny, C., and Foster, T. L.: J. Kansas M. Soc. 57:410, 1956.
2. Terman, L. A.: Illinois M.J. 3:67, 1957.
3. Moyer, J. H.; Dennis, E., and Ford, R.: Arch. Int. Med. 96:530, 1955.

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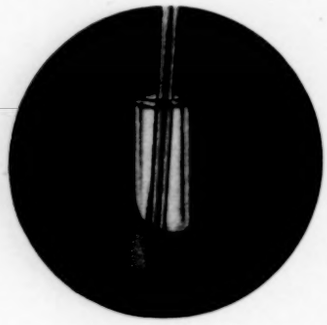
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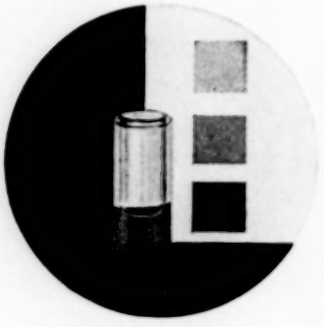
2. Crush tablet.



3. Let stand at room temperature 12-30 minutes. (time from chart)



4. Add 1 drop of color developer: mix.



5. Compare color.

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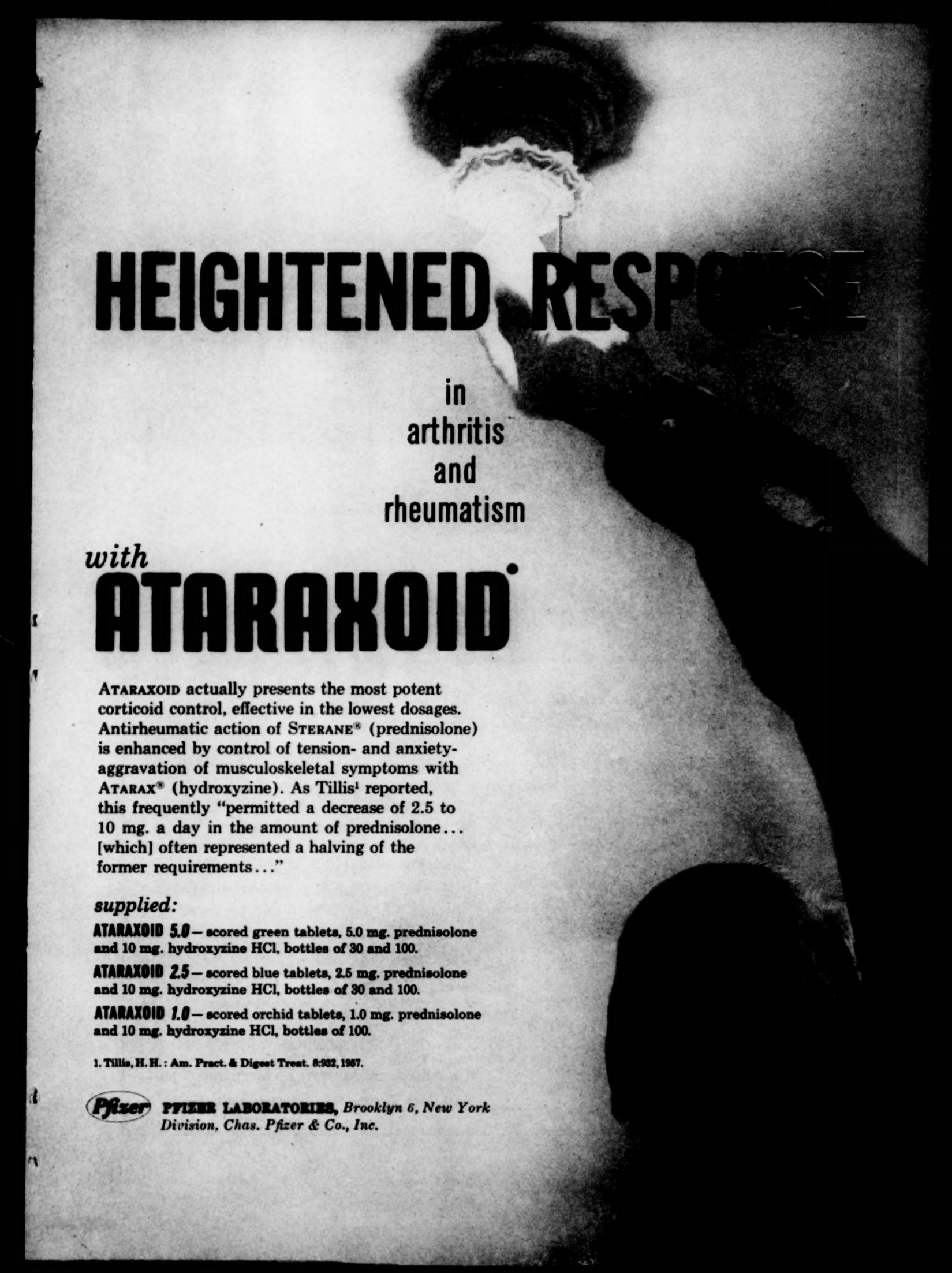
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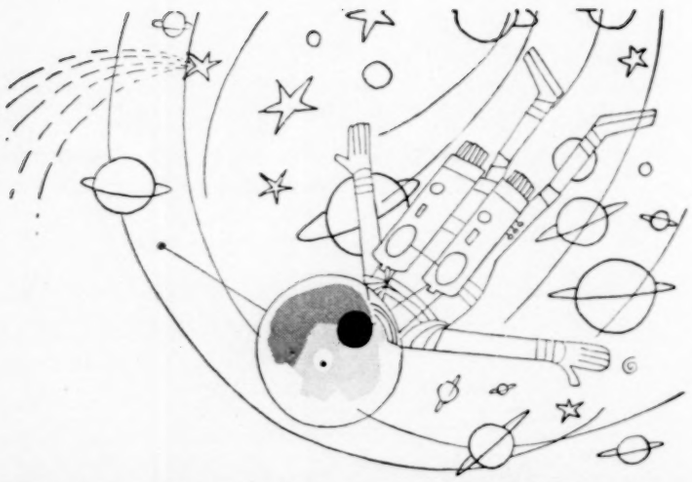
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1. Tillis, H. H.: Am. Pract. & Digest Treat. 8:932, 1957.

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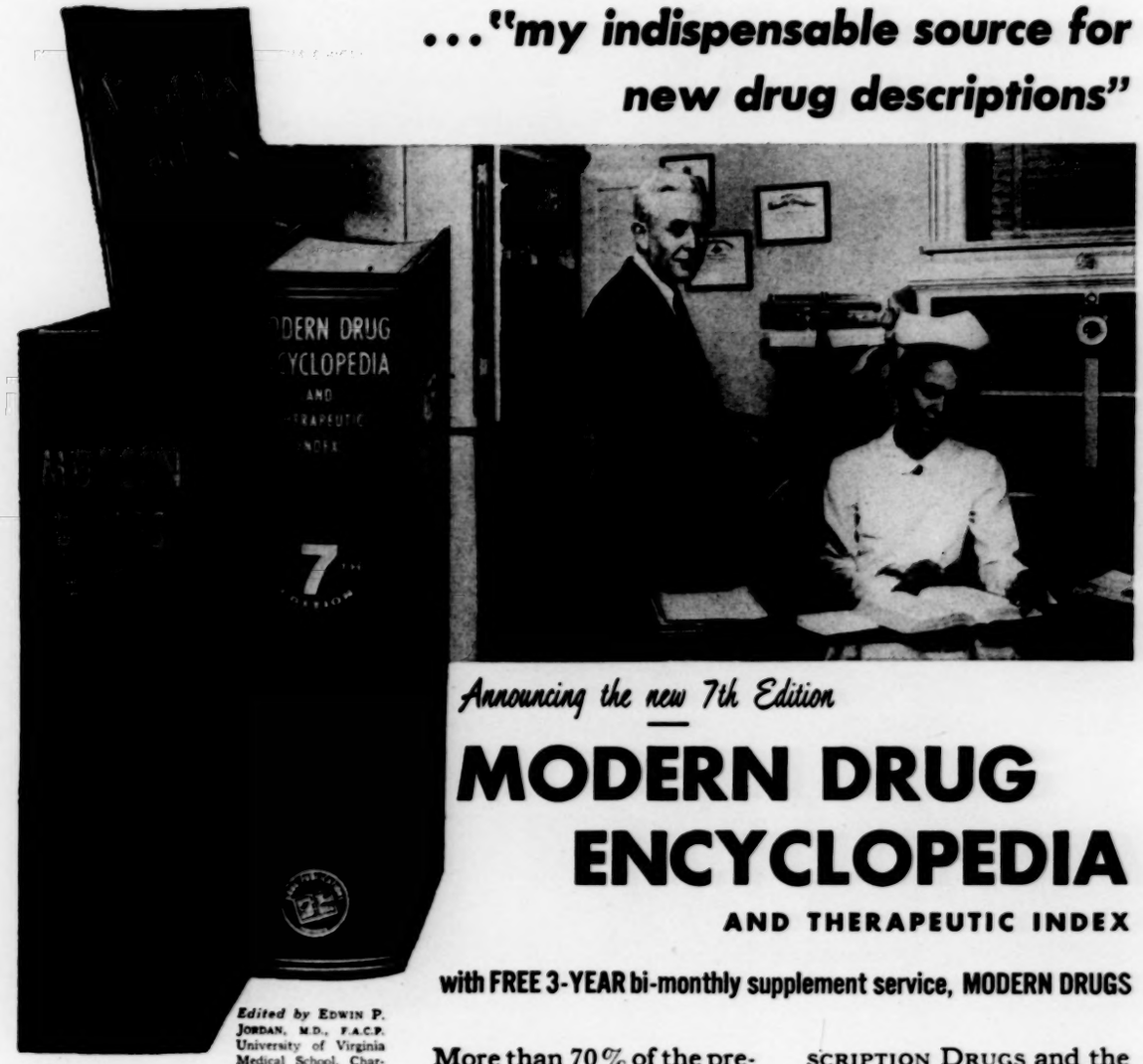
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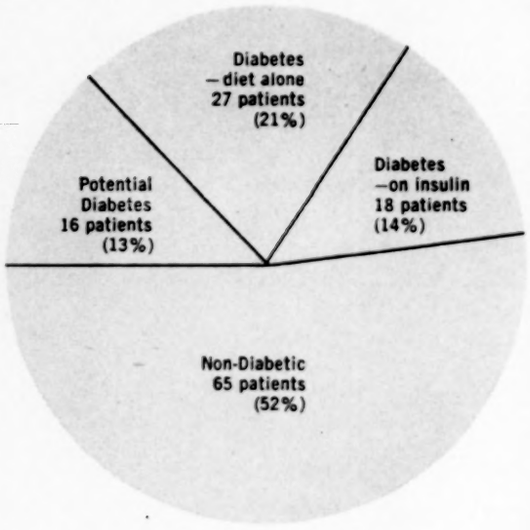
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